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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

AUG 4 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: DEET: Review of a chronic toxicity/oncogenicity study in rats, a 8-week dietary feeding dose-range finding study in dogs, and a 9-week oral dose-range finding study in dogs (gelatin capsule)

Caswell No.	346	**MRID No.**	43514201 43514202 43514203
PC Code.	080301	**DP Barcode:**	D211262
Submission No.	S480555	**EPA ID No.**	N80301-051147

TO: Jane Mitchell / Walter Waldrop, PM Team 71
Special Review and Re-registration Division (7508C)

FROM: Whang Phang, Ph.D. 8/1/95
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THROUGH: James Rowe, Ph.D. James N. Rowe 8/2/95
Section Head, Section III
and
Karl Baetcke, Ph.D. 8/3/95
Acting Branch Chief
Tox. Branch II/ HED (7509C)

Toxicology Branch II has been requested to review a chronic/ oncogenicity study in rat, a 8-week dietary feeding dose-range finding study in dogs, and a 9-week oral dose-range finding study in dogs (gelatin capsule). These studies have been reviewed. The DER for each study is attached, and the citation for each study and conclusion of the each review are the following:

1. **Citation:** Goldenthal, E. I. (1995) Evaluation of DEET in a two-year dietary and oncogenicity study in rats. Unpublished study conducted by IRDC. Study No. 555-023. January 3, 1995. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 43514203.

Conclusion: Groups of CD^R rats (60 sex/dose) received DEET (98.3% purity) in the diet at dose levels of 10, 30, and 100 mg/kg for male and 30, 100, and 400 mg/kg for females. Two control groups were run concurrently. The animals were treated for 2 years. The findings are summarized as follows:

a. There was a decrease in the body weights of 400 mg/kg females. The decrease was progressive with length of the study (≈9% at 26 week and nearly 18% by 104 week) and showed statistical significance.

b. Food consumption in the 400 mg/kg females was decreased (8%) relative to the controls.

c. There was a statistically significant increase (≈25 to 50%) in cholesterol levels in 400 mg/kg females relative to the controls at various measuring intervals.

d. No compound-related increases in non-neoplastic or neoplastic lesions were seen.

Based on results of this study, the NOEL for the chronic toxicity of DEET in **females** is 100 mg/kg; LEL, 400 mg/kg (decreased body weights and food consumption and increased cholesterol levels in female rats). No toxicity was seen in any dose groups of male rats. The NOEL for the chronic toxicity of DEET in **males** is 100 mg/kg (HDT).

A treatment-related increase in tumor incidence was not seen.

This study is classified as **minimum with respect to female rats**, and meets the data requirements for a combined chronic toxicity/oncogenicity study in **female rats** (83-5). With respect to male rats, the test animals clearly could have tolerated higher doses. However, it is unclear why kidney lesions were not observed in the 2-year study since they were seen at equivalent or lower dosages in the 90-day and reproduction studies. This may be due to a physiological adaptation mechanism.

2. **Citation:** Goldenthal, E.I. (1994) Evaluation of DEET in an eight week oral gelatin capsule toxicity study in dogs. International Research and Development Corp.; Study No. 555-027. January 3, 1995. Submitted to EPA by CSMA. EPA MRID No. 43514201

Conclusion: In a 8-week dose-range finding study, groups of beagle dogs (2/sex/dose) received DEET in a gelatin capsule at dose levels of 50, 100, 200, or 400 mg/kg/day. The control animals received white mineral oil in gelatin capsule. The

following results were obtained:

1. Clinical observation data showed a significant increase in ptyalism in 100 mg/kg or above males and females and an increase in abnormal head movements in 400 mg/kg males.

2. A decrease in body weight gains was found in 400 mg/kg males and females, and that in female dogs was more marked.

3. Food consumption was substantially reduced in 400 mg/kg females.

4. There was a decrease in cholesterol level in 400 mg/kg male dogs.

5. A decrease in testis/epididymis weight was found in 400 mg/kg males. However, both gross examination and histopathology did not indicate any changes in the testis or any other organs.

The reliability of the results of this study is compromised by the small number of dogs (2/sex/dose) used, and a useful NOEL and LEL could not be established.

Based on the above results, the registrant selected 400 mg/kg as the highest dose and 30 and 100 mg/kg as low and mid dose, respectively, for a chronic toxicity study in dogs. The selected doses for the chronic toxicity appeared to be adequate.

This study is classified as **supplementary**, and does not meet the data requirements for a subchronic oral toxicity study in dogs (82-1).

3. **Citation:** Goldenthal, E.I. (1994) Evaluation of DEET in an eight week dietary toxicity study in dogs. International Research and Development Corp.; Study No. 555-020. January 3, 1995. Submitted to EPA by CSMA. EPA MRID No. 43514202

Conclusion: In an 8-week dose-range finding study, groups of beagle dogs (2/sex/dose) received DEET in the diet at concentrations of 300, 1000, 3000, or 6000/4500/3000 ppm[1]

[1] During the first two weeks of the study, the highest dose male and female dogs received 6000 ppm test diet, but the dogs rejected the test diet. The treatment diet was withdrawn at the end of the second week, and the animals were given the basal diet for ≈1 week. The dosage was then reduced at week 4 from 6000 ppm to 4500 ppm and at week 7 from 4500 ppm to 3000 ppm. At week 6, this dose group of dogs was again given the basal diet.

(8.4, 28.6, 93.3, or 19.5 mg/kg for males and 9.7, 30.6, 91.8, or 11.5 mg/kg for females). The control animals received basal diet.

Under the conditions of this study, DEET did not produce any toxicity at dietary concentrations of 3000 ppm or less. At a concentration of 6000/4500/3000 ppm, DEET caused food rejection which led to a decrease in body weight, thin appearance, fat depletion, organ weight decrease, and histological changed in kidneys, bone marrow, and thymus.

The reliability of the results of this study is compromised by the small no of dogs (2/sex/dose) used, and a useful NOEL and LEL could not be established.

This study is classified as **supplementary**, and does not meet the data requirements for subchronic oral toxicity study in dogs (82-1)

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Reviewer: Whang Phang, Ph.D. Whang Phang 8/1/95
Tox. Branch II (7509C)

Secondary Reviewer: James Rowe, Ph.D. James N. Rowe 8/1/95
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DATA EVALUATION REPORT

Study Type: Two-year chronic toxicity/oncogenicity study in rats (§83-5)

Chemical: DEET (N, N-diethyl-m-toluamide)

Caswell No.	346	**MRID No.**	43514203
PC Code.	080301	**DP Barcode:**	D211262
Submission No.	S480555	**EPA ID No.**	N80301-051147

Sponsor: DEET Joint Venture/Chemical Specialties Manufacturers Association

Testing Facility: International Research and Development Corp.
500 North Main St.
Mattawan, Michigan 49071

Citation: Goldenthal, E. I. (1995) Evaluation of DEET in a two-year dietary and oncogenicity study in rats. Unpublished study conducted by IRDC. Study No. 555-023. January 3, 1995. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 43514203.

Conclusion: Groups of CD^R rats (60 sex/dose) received DEET (98.3% purity) in the diet at dose levels of 10, 30, and 100 mg/kg for male and 30, 100, and 400 mg/kg for females. Two control groups were run concurrently. The animals were treated for 2 years. The findings are summarized as follows:

a. There was a decrease in the body weights of 400 mg/kg females. The decrease was progressive with length of the study (≈9% at 26 week and nearly 18% by 104 week) and showed statistical significance.

b. Food consumption in the 400 mg/kg females was decreased (8%) relative to the controls.

c. There was a statistically significant increase (≈25 to 50%) in cholesterol levels in 400 mg/kg females relative to the controls at various measuring intervals.

d. No compound-related increases in non-neoplastic or neoplastic lesions were seen.

Based on results of this study, the NOEL for the chronic toxicity of DEET in **females** is 100 mg/kg; LEL, 400 mg/kg

(decreased body weights and food consumption and increased cholesterol levels in female rats). No toxicity was seen in any dose groups of male rats. The NOEL for the chronic toxicity of DEET in **males** is 100 mg/kg (HDT).

A treatment-related increase in tumor incidence was not seen.

This study is classified as **minimum with respect to female rats**, and meets the data requirements for a combined chronic toxicity/oncogenicity study in **female rats** (83-5). With respect to male rats, the test animals clearly could have tolerated higher doses. However, it is unclear why kidney lesions were not observed in the 2-year study since they were seen at equivalent or lower dosages in the 90-day and reproduction studies. This may be due to a physiological adaptation mechanism.

For appropriateness of the doses tested, please see the DISCUSSION section.

Methods and Materials

Test Article: DEET Insect Repellent (N,N-diethyltoluamide; 98.301% purity). The test article was a mixture of equal parts of 4 representative production runs supplied by McLaughlin Gormley King Co. (Lot No. 10111), Miles Laboratories (Lot No. 90003), Virginia Chemical Co. (Lot No. 85227), and Morflex Chemical Co. (Lot No. N61214-59401). The test chemical was described as a clear liquid, with a Lot No. of A-1-96, and a IRDC No. of 8812B.

Test Animals: Five week old Charles River CD^R rats were obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan. The test animals were acclimated to the laboratory conditions for 3 weeks prior to the initiation of the study.

Study design

1. Dose selection: The dosages (**0, 10, 30, & 100 mg/kg for males and 0, 30, 100, & 400 mg/kg for females**) for this study were selected based on the results of a 90-day feeding study in rats (MRID No. 42041703) and a two-generation reproduction study in rats (MRID No. 40979001).

   In the 90-day study, the groups of CD^R rats (15/sex/dose) received DEET in the diet at doses of 100, 500, 1000, 2000, and 4000 mg/kg. An increase in the incidence of kidney lesions (characterized by granular casts, inflammation, regeneration, and presence of hyaline droplets) were seen in all treated males. In addition, a decrease in mean body weight, body weight gain, and food consumption were found in

males and females at 500 mg/kg or above.

In the 2-generation reproduction study in rats, Sprague Dawley rats received DEET at dietary concentrations of 500, 2000, and 5000 ppm. Kidney lesions (characterized by inflammation, presence of hyaline droplets and granular casts, congestion and regeneration of the tubules) were seen in all treated males. The 500 ppm dietary level was approximately equivalent to 35 mg/kg. At 5000 ppm (approximately equal to 350 mg/kg), there was a decrease in body weight and food consumption in the F_0 females.

The report also stated that in considering the dose selection, a pathologist, Donald N. Kitchen, who specialized in the area of renal pathology, was consulted. Dr. Kitchen examined the renal lesions of both 90-day and the 2-generation reproduction study. He concluded that the findings of the kidney lesions in the 100 mg/kg males in the 90-day study and in 500 ppm (≈ 35 mg/kg) and 2000 ppm males in the 2-generation reproduction were of similar origin and severity. Therefore, 100 mg/kg was selected as the highest dose for males in the chronic study. Based on decreases in food consumption and body weights in 5000 ppm (≈350 mg/kg) females in the reproduction study, 400 mg/kg was selected as the highest dose for females (please see DISCUSSION section concerning the Agency's decision on the dosage selection).

2. Test animal assignments: Three hundred males (weighing from 273 to 325 gm) and 300 femalef (weighing from 190 to 224 gm) were randomly assigned to the following test groups;

Dose levels (mg/kg) Male / Female	Number of Animals Male	Female
0 (Control 1)	60	60
10 / 30	60	60
30 / 100	60	60
100 / 400	60	60
0 (Control 2)*	60	60

*: The purpose for including 2 control groups in this study was to collect data to provide information on the range of normal or control values for evaluated parameter (For more details, please see Appendix A).

3. Test diet preparation and administration: The test diet was prepared from a concentrated premix, which was prepared by adding appropriate amounts of DEET to the diet and blended thoroughly. After preparation, samples of the test diets were taken for determination of stability, homogeneity, and targeted concentration. The test animals were offered feed and water ad. lib.

4. Clinical observations: The test animals were observed twice daily for toxicity, moribundity, and mortality.

5. Body weights: Body weights of the test animals were measured weekly during the first 14 weeks of the study and once every two weeks thereafter.

6. Food consumption: Food consumption was determined weekly during the first 14 weeks of the study and once every two weeks thereafter.

7. Ophthalmology: Eye examinations were conducted on each rat at pretest and in the last week of the study.

8. Hematology: Blood samples were obtained from 15 randomly selected animals/sex/dose group at 6, 12, 18 and 24 months of the study. The selected animals were fasted for 24 hours prior to sample collection. The blood samples were used for hematology and clinical chemistry. The following hematological parameters were analyzed:

erythrocyte count	hemoglobin
leukocyte count	differential leukocyte count
hematocrit	platelet
reticulocyte count	Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)	Mean corpuscular hemoglobin concentration (MCHC)

9. Clinical chemistry: The following biochemistry parameters were determined:

sodium	potassium
chloride	calcium
phosphorus	total bilirubin
aspartate aminotransferase (AST) (SGOT)	alanine aminotransferase (ALT) (SGPT)
urea nitrogen	creatinine
total protein	albumin
globulin	glucose
alkaline phosphatase	creatine phosphokinase (CPK)
cholesterol	direct & indirect bilirubin
albumin/globulin (A/G) ratio	

10. Urinalysis: Urine samples were collected during the fasting period, and the following parameters were examined:

color and appearance	volume
specific gravity	microscopic elements
pH	protein
glucose	ketones
bilirubin	occult blood
nitrites	urobilinogen
leukocytes	

11. Pathology: At end of 2 years of treatment, all animals were weighed and sacrificed with carbon dioxide over a 6-day period with animals from each group euthanized on each day.

    a. Necropsy: A thorough postmortem examination was conducted on each animal. The abdominal, thoracic, and cranial cavities were specifically examined for abnormalities.

    b. Organ weights: The following organs were removed, trimmed free of fat, and weighed:

adrenals	liver
brain with stem	ovaries
kidneys	testis
heart	spleen

    c. Histopathology: The following organs were removed, placed in the phosphate-buffered neutral formalin, and processed for microscopic examination.

adrenal	kidney (2)
aorta	liver
bone (femur & sternum)	bone marrow (femur & sternum)
lung with bronchi	heart
bone marrow & smears	lymph nodes (tracheobronchial,
brain	mesenteric, mandibular)
eye with optic nerve	mammary gland
gallbladder	pancreas
GI tract:	pituitary
esophagus	prostate & seminal vesicles
stomach	salivary gland
duodenum	sciatic nerve
jejunum	skeletal muscle (thigh)
ileum	skin
cecum	spinal cord
colon	spleen
rectum	tissue masses
ovary	thymus
testes with epididymis	thyroid/parathyroid
trachea	urinary bladder
uterus/horns/cervix	gross lesions
vagina	

    A grading system consisting of trace, mild, moderate, and severe was used to define any gradable lesions for comparison purposes.

12. Statistics: The details of statistical analysis methods were excerpted from the report and presented in Appendix A (p.15).

13. Quality assurance: A statement of no data confidentiality claim, a statement of compliance, a flagging statement, and a quality assurance statement were signed and included in the report.

RESULTS

1. Test diet analysis: The analysis of the test article indicated that the test diet formulations were uniform and that the test substance was stable in the diet for 2 weeks at room temperature. In addition, periodic concentration analysis indicated that diet formulations were generally within an average of 1-2% of the nominal concentration.

2. Clinical signs: No compound-related toxic signs were seen.

3. Mortality: There was a slight decrease in the survival rate in 30 and 100 mg/kg males, but a dose related effect was not present (Table 1). Therefore, the survival rates between the treated and control animals were considered to be comparable.

Table 1+. Survival Rate at 105 Weeks (No. survivor/No. on test)

mg/kg (M/F)	0 (Con.1)	0 (Con.2)	10/30	30/100	100/400
Males	31/60	30/60	25/60	22/60	23/60
Females	19/60	23/60	16/60	28/60	23/60

+: Data excerpted from the report, p. 37-56 (MRID No.43514203).

4. Body weights: Body weights of males were comparable between the treated and the control groups. There was a statistically significant decrease in body weight of the 400 mg/kg females relative to the two control groups (Table 2), and this decrease was greater as the study progressed (approximately 9% at 26 week and nearly 17-19% by 104 weeks).

5. Food consumption: Food consumption in the treated and control males was comparable. Based upon calculation of food consumption (g/animal/day) through 104 weeks of treatment, there was approximately an 8% decrease in food consumption in the 400 mg/kg females relative to either of the two female control groups.

6. Ophthalmological examination: No compound-related eye abnormalities were found.

Table 2+. Mean Body Weights (g)

mg/kg (M/F)	0 (Con.1)	0 (Con.2)	10/30	30/100	100/400
26 Weeks (g)					
Males	684	689	683	694	684
Females	385	389	382	376	352(9%)**
52 Weeks (g)					
Males	789	793	786	793	770
Females	463	465	461	442	405** (13%)
78 Weeks (g)					
Males	826	826	839	826	788
Females	499	512	517	499	417** (16%-19%)
104 Weeks (g)					
Males	809	758	740	751	740
Females	540	525	495	529	435* (17%-19%)

*: Significantly different from the controls ($p \leq 0.05$).

**: Significantly different from the controls ($p \leq 0.01$)

(): % decrease relative to the controls.

+: Data excerpted from the report; p. 25 (MRID No. 43514203).

7. Hematology: No treatment-related hematological changes were found.

8. Biochemistry: There was an increase in cholesterol levels in the 400 mg/kg females at 6, 12, and 18 months and at terminal sacrifice (Table 3). These increases were statistically significant at most measuring intervals except the terminal sacrifice. The increases ranged from 26% to 50%. There were sporadic changes in other biochemistry parameters, but they were not compound-related changes.

9. Urinalysis: There were no treatment-related changes in the parameters of urinalysis.

10. Gross pathology: A compound-related increase in gross pathological findings was not seen.

Table 3[+]. Mean cholesterol level (mg/dl) in DEET treated female rats

Month of study	0 mg/kg (Cont.1)	30 mg/kg	100 mg/kg	400 mg/kg	0 mg/kg (Cont.2)
6	78±15	88±21	92±17	101±25[1,3]	80±11
12	74±19	97±24[1]	94±25[1]	114±21[2,4]	89±21
18	72±19	81±19	83±19	105±27[2,4]	70±20
24	120±47	128±55	118±38	149±63	119±90

+: Data excerpted from the report; p. 138 & 139 (MRID No.43514203).
1: Significantly different from control group 1; $p \leq 0.05$.
2. Significantly different from control group 2; $p \leq 0.01$.
3. Significantly different from control group 1; $p \leq 0.05$.
4. Significantly different from control group 2; $p \leq 0.01$.

11. Organ weights: The absolute and relative organ weights of the treated males were comparable to those of the controls. The absolute organ weights of the females were also comparable to those of the controls. However, in 400 mg/kg females, there was a statistically significant increase in relative brain/body weight (%x10)(Control 1, 4.02; Control 2, 4.18; 400 mg/kg, 4.86) and relative liver/body weights (%)(Control 1,3.88; Control 2, 4.04; 400 mg/kg, 5.14). The increase in relative liver and brain weights was primarily due to a decrease in body weights.

12. Histopathology : A number of histopathological changes were reported in the test animals across all dose groups (Table 4). These findings did not show a dose-related effect, and they were not compound related. There was a slight increase in the incidence of renal cell adenoma in 10 mg/kg male rats (Control 1, 1/60; Control 2, 0/60; 10 mg/kg, 3/60). However, no renal cell adenoma was seen in either 30 or 100 mg/kg males (Table 4; p. 11-14). This incidence was not considered as a compound-related effect due to lack of dose-response relationship, and since a similar tumor was also seen in one control male.

DISCUSSION

Groups of CD^R rats (60 sex/dose) received DEET (98.3% purity) in the diet at dose levels of 10, 30, and 100 mg/kg for male and 30, 100, and 400 mg/kg for females. Two control groups were run concurrently. The animals were treated for 2 years. The findings are summarized as follows:

a. No increase in the clinical signs of toxicity were found

in treated animals relative to the two control groups.

b. The survival rates between the treated and the control groups were comparable.

c. The body weights in treated males were comparable to the controls, while those in the 400 mg/kg females was decreased. The decrease was progressive with the length of time on study (≈9% at 26 week and nearly 18% by 104 week) and showed statistical significance.

d. Food consumption in the 400 mg/kg females was also decreased (8%) relative to the controls over the study duration.

e. There was a statistically significant increase (≈25 to 50%) in cholesterol levels in 400 mg/kg females relative to the controls at various measuring intervals.

f. The absolute organ weights were comparable to the controls. There was an increase in relative brain weight (brain/body weight) and relative liver weight (liver/body weight) in 400 mg/kg females, but this increase was mainly due to decreased body weight.

g. Compound-related increases in the incidences of non-neoplastic or neoplastic lesions were not seen.

As indicated by the results of this study, no renal lesions were found in the treated male rats even at the highest dose (100 mg/kg). In contrast, in the 90-feeding study and the 2-generation reproduction study, renal lesions (characterized by the inflammation, regeneration and the presence of granular casts and hyaline droplets in the kidney tubules) were found in all treated males at doses as low as 500 ppm (≈35 mg/kg). The renal lesions in DEET treated male rats were later shown to be related to α-2μ-globulin nephropathy. There are two questions which must be explored: (1) Is the highest dose (100 mg/kg) tested in male rats high enough? (2) Is the strain of rats used in this study resistant to DEET induced renal lesions?

In considering whether or not the highest tested dose (100 mg/kg) was high enough for male rats, the record showed that prior to the initiation of the study, the registrant had consulted with the Agency concerning the dosage selection for this study. At the beginning, the Agency felt that the highest dose (100 mg/kg) for male rats might not be high enough (Memorandum, W. Phang to J. Mitchell, July 11, 1991). A meeting was held, and attended by the representatives of HED, RD (PM 10), and the registrant. During the discussion Dr. K. Baetcke presented the Agency's concern that male rats

might be able to tolerate higher doses than 100 mg/kg as proposed because "α-2μ-globulin nephropathy does not, in and of itself, result in life-threatening kidney toxicity as demonstrated by the d-limonene bioassay where survival of the high dose (150 mg/kg) was excellent (Control, 60%; high dose, 80%) while the incidence of renal lesions was significantly greater than the controls". The toxicologists representing the registrant presented their rationale (see beginning of the Study Design section) for selecting 100 mg/kg as the highest dose for male rats. After much discussion, the Agency reluctantly conceded that the registrant may employ 100 mg/kg as the highest dose for male rats in the chronic/ oncogenicity study. The results of this present study clearly showed that male rats could have tolerated higher doses than 100 mg/kg.

In considering the second question, is the strain of rats used in this study resistant to DEET induced renal lesions, the registrant submitted a 90-day feeding study in multiple strains of rats (CD^R, Fischer, and NBR) (MRID No. 42518101; Tox. Doc. No. 010528). The results showed that CD^R and Fischer rats are susceptible while NBR rats are resistant to DEET induced renal lesions. Based on the available information, the chronic/oncogenicity study employed appropriate strain of rats (CD^R) for testing.

Based on these considerations and the results of this study, the NOEL for the chronic toxicity of DEET in **females** is 100 mg/kg; LEL, 400 mg/kg (decreased body weights and food consumption and increased cholesterol levels in female rats). No toxicity was seen in any dose groups of male rats. The NOEL for the chronic toxicity of DEET in **males** is 100 mg/kg (HDT).

A treatment-related increase in tumor incidence was not seen.

This study is classified as **minimum with respect to female rats**, and meets the data requirements for a combined chronic toxicity/oncogenicity study in **female rats** (83-5). With respect to male rats, the test animals clearly could have tolerated higher doses.

Table 4*

Incidence of Microscopic Observations
Day 0 through Terminal Sacrifice: Rat
Male

TISSUE OBSERVATION		0 mg/kg/day (Control 1)		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		0 mg/kg/day (Control 2)	
		DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Epididymis		(29)	(31)	(4)	(0)	(2)	(0)	(37)	(23)	(31)	(29)
Within normal limits		26	29	4	0	0	0	33	21	26	25
Atrophy, moderate		1	0	0	0	0	0	0	0	0	0
Sarcoma, histiocytic		0	0	0	0	1	0	0	0	0	0
Lumenal debris, cellular,		1	2	0	0	0	0	4	2	5	4
	-trace	0	0	0	0	0	0	1	0	1	1
	-mild	1	2	0	0	0	0	3	2	4	3
Metastatic tumor present		0	0	0	0	1	0	0	0	0	0
Periarteritis, mild		1	0	0	0	0	0	0	0	0	0
Kidney		(29)	(31)	(36)	(24)	(38)	(22)	(37)	(23)	(31)	(29)
Within normal limits		3	3	6	1	0	0	4	0	3	3
Cyst,		0	1	1	3	1	2	3	3	0	4
	-trace	0	0	0	1	0	0	0	1	0	0
	-mild	0	1	1	1	1	2	1	0	0	4
	-moderate	0	0	0	1	0	0	2	1	0	0
	-severe	0	0	0	0	0	0	0	1	0	0
Inflammation, chronic, mild		0	0	0	1	0	0	1	0	0	0
Mineralization, mild		1	0	2	2	2	0	0	0	0	0
Congestion, mild		1	0	0	0	0	0	0	0	1	0
Hydronephrosis,		1	3	1	0	1	1	3	0	1	1
	-mild	1	1	1	0	1	1	1	0	0	1
	-moderate	0	2	0	0	0	0	2	0	1	0
Inclusion, cytoplasmic, mild		0	0	0	0	1	0	0	0	0	0
Infarct, mild		1	0	0	0	0	0	0	0	0	0
Leukemia, mononuclear cell		2	0	0	0	0	0	0	1	0	1
Lipoma		0	0	0	0	1	0	0	0	0	0
Mesenchymal tumor, malignant		0	0	1	0	0	0	0	0	0	0
Metastatic tumor present		0	0	2	0	0	0	0	0	0	0
Nephropathy, chronic progressive,		23	28	28	22	36	22	32	23	26	25
	-trace	0	3	5	8	9	6	6	2	2	4
	-mild	18	20	18	5	15	10	18	16	15	13
	-moderate	3	5	5	7	7	5	6	3	5	5
	-severe	2	0	0	2	5	1	2	2	4	3
Adenoma, renal cell		0	1	3	0	0	0	0	0	0	0
Thrombosis, mild		0	0	0	0	1	0	0	0	0	0
Liver		(29)	(31)	(36)	(24)	(38)	(22)	(37)	(23)	(31)	(28)
Within normal limits		8	8	12	8	6	8	12	4	8	6
Cyst, biliary,		1	1	0	0	0	1	1	2	0	0
	-mild	1	1	0	0	0	0	1	2	0	0
	-moderate	0	0	0	0	0	1	0	0	0	0
Inflammation, chronic,		1	0	0	0	1	2	2	2	2	3
	-trace	1	0	0	0	1	2	1	2	1	1
	-mild	0	0	0	0	0	0	1	0	1	2
Necrosis,		1	1	0	0	5	0	3	0	1	0
	-trace	0	0	0	0	2	0	0	0	0	0
	-mild	1	1	0	0	1	0	1	0	1	0
	-moderate	0	0	0	0	1	0	1	0	0	0
	-severe	0	0	0	0	1	0	1	0	0	0
Vacuolar change,		5	3	0	1	6	0	6	2	10	6
	-trace	3	2	0	1	1	0	1	1	5	4
	-mild	2	1	0	0	4	0	3	1	4	1
	-moderate	0	0	0	0	1	0	2	0	1	1
Adhesion, mild		0	0	1	0	0	0	0	0	0	0
Altered foci, basophilic, mild		0	0	1	0	0	1	2	0	0	0
Altered foci, clear cell,		1	2	0	1	1	2	1	4	2	1
	-trace	0	0	0	0	0	0	0	1	0	0
	-mild	0	2	0	1	1	2	1	3	2	1
	-moderate	1	0	0	0	0	0	0	0	0	0
Altered foci, eosinophilic, mild		1	0	0	1	0	2	0	1	0	1
Cholangioma		0	0	0	0	0	0	0	1	0	0
Congestion,		11	16	13	8	10	8	12	14	10	9
	-trace	1	3	1	6	0	8	0	10	2	1
	-mild	10	13	11	2	9	0	11	4	7	8
	-moderate	0	0	1	0	1	0	1	0	1	0
Fibrosis, moderate		0	0	0	0	1	0	0	0	0	0
Adenoma, hepatocellular		1	0	1	2	1	1	2	0	0	0
Carcinoma, hepatocellular		1	2	2	0	4	1	0	0	1	3
Sarcoma, histiocytic		1	0	0	0	2	0	1	0	1	1
Hyperplasia, bile duct,		5	3	4	2	3	2	3	5	3	3
	-trace	1	3	2	2	2	1	1	4	1	2
	-mild	4	0	2	0	1	1	2	1	2	1
Hypertrophy, mild		1	0	0	0	0	0	0	0	0	0
Leukemia, mononuclear cell		2	0	2	0	1	0	2	1	0	1
Metastatic tumor present		0	0	1	0	0	0	0	0	0	0
Spongiosis hepatis,		3	7	7	8	9	5	8	6	1	6
	-trace	1	4	3	1	2	0	3	1	0	4
	-mild	1	3	4	5	7	5	5	5	1	2
	-moderate	1	0	0	0	0	0	0	0	0	0
Telangiectasis,		2	1	2	0	1	3	0	1	1	3
	-trace	0	0	0	0	0	0	0	0	0	2
	-mild	2	1	2	0	1	3	0	0	0	1
	-moderate	0	0	0	0	0	0	0	1	1	0

555-023

CODE: () = NUMBER OF ANIMALS EXAMINED

*: Data excerpted from the report, p. (MRID No. 43514203).

Table 4 Cont.

Incidence of Microscopic Observations
Day 0 through Terminal Sacrifice: Rat
Male

TISSUE OBSERVATION		0 mg/kg/day (Control 1)		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		0 mg/kg/day (Control 2)	
		DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Prostate Gland		(29)	(31)	(9)	(2)	(7)	(1)	(37)	(23)	(31)	(29)
Within normal limits		7	29	4	0	0	0	29	19	16	20
Inflammation, chronic,		13	0	1	0	0	0	6	3	2	9
	-trace	1	0	0	0	0	0	0	1	0	1
	-mild	10	0	1	0	0	0	5	2	2	6
	-moderate	1	0	0	0	0	0	1	0	0	2
	-severe	1	0	0	0	0	0	0	0	0	0
Mineralization, mild		0	0	0	0	0	0	1	0	0	0
Abscess,		5	2	3	1	6	1	2	0	13	2
	-mild	1	1	1	0	4	1	0	0	6	0
	-moderate	3	1	2	1	2	0	2	0	5	1
	-severe	1	0	0	0	0	0	0	0	2	1
Leukemia, mononuclear cell		2	0	0	0	0	0	0	0	0	0
Lumenal debris, cellular, mild		2	0	1	1	0	0	0	1	0	0
Metastatic tumor present		0	0	0	0	1	0	0	0	0	0
Testis		(29)	(31)	(10)	(1)	(11)	(2)	(37)	(23)	(31)	(29)
Within normal limits		20	28	3	0	3	0	31	21	23	25
Atrophy, mild		0	0	0	0	0	0	1	0	0	0
Mineralization,		0	0	0	0	1	0	0	0	1	0
	-trace	0	0	0	0	0	0	0	0	1	0
	-moderate	0	0	0	0	1	0	0	0	0	0
Degeneration, seminiferous tubules,		4	3	5	1	4	1	4	0	7	4
	-mild	0	1	2	1	1	0	2	0	3	2
	-moderate	3	1	3	0	2	1	2	0	3	2
	-severe	1	1	0	0	1	0	0	0	1	0
Congestion,		1	0	0	0	3	0	0	0	0	0
	-trace	0	0	0	0	1	0	0	0	0	0
	-mild	1	0	0	0	2	0	0	0	0	0
Sarcoma, histiocytic		0	0	0	0	0	0	1	0	0	0
Leukemia, mononuclear cell		1	0	0	0	0	0	0	0	0	0
Leydig cell tumor, benign		4	0	1	0	1	1	0	2	0	0
Periarteritis,		1	0	1	0	1	0	0	0	0	1
	-mild	0	0	1	0	0	0	0	0	0	1
	-moderate	1	0	0	0	1	0	0	0	0	0
Thymic Region		(29)	(31)	(6)	(0)	(1)	(1)	(36)	(23)	(31)	(29)
Within normal limits		23	31	4	0	1	0	33	22	29	29
Cyst, severe		0	0	0	0	0	1	0	0	0	0
Hemorrhage, moderate		1	0	0	0	0	0	0	0	0	0
Thymus not in plane of section		1	2	0	0	0	0	2	0	3	1
Congestion, mild		3	0	0	0	0	0	0	0	0	0
Fibrosarcoma		0	0	1	0	0	0	1	0	0	0
Sarcoma, histiocytic		0	0	0	0	0	0	1	0	1	0
Hyperplasia, lymphoid, moderate		0	0	0	0	0	0	0	1	0	0
Leukemia, mononuclear cell		2	0	1	0	0	0	1	0	0	0
Metastatic tumor present		0	0	1	0	0	0	0	0	1	0

555-023 CODE: ( ) = NUMBER OF ANIMALS EXAMINED

+: Data excerpted from the report, p. (MRID No. 43514203).

Table 4 Cont.

Incidence of Microscopic Observations
Day 0 through Terminal Sacrifice: Rat
Female

TISSUE / OBSERVATION		0 mg/kg/day (Control 1)		30 mg/kg/day		100 mg/kg/day		400 mg/kg/day		0 mg/kg/day (Control 2)	
		DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Kidney		(43)	(17)	(44)	(16)	(32)	(28)	(37)	(23)	(37)	(23)
Within normal limits		13	4	19	4	13	6	9	10	11	7
Cyst,		0	0	0	0	1	0	0	1	1	0
	-trace	0	0	0	0	0	0	0	0	1	0
	-mild	0	0	0	0	1	0	0	0	0	0
	-moderate	0	0	0	0	0	0	0	1	0	0
Inflammation, chronic, mild		0	0	0	0	1	0	0	0	0	0
Mineralization,		13	1	6	2	3	2	8	1	10	5
	-trace	5	0	6	1	0	1	6	1	5	5
	-mild	8	1	0	1	3	1	2	0	5	0
Necrosis, moderate		0	0	0	0	0	0	1	0	0	0
Congestion, mild		2	0	0	0	0	0	1	0	0	0
Ectopic tissue, mild		0	0	0	0	0	0	1	0	0	0
Sarcoma, histiocytic		0	0	0	0	1	0	0	0	0	0
Hydronephrosis,		1	0	0	0	1	0	1	0	0	1
	-trace	0	0	0	0	0	0	1	0	0	0
	-mild	1	0	0	0	1	0	0	0	0	1
Inclusion, cytoplasmic,		0	0	2	0	0	0	0	0	0	0
	-mild	0	0	1	0	0	0	0	0	0	0
	-moderate	0	0	1	0	0	0	0	0	0	0
Leukemia, mononuclear cell		1	0	1	0	1	0	0	0	0	1
Lipoma		0	0	0	1	0	0	0	0	0	1
Nephropathy, chronic progressive,		21	13	21	9	16	20	26	11	20	15
	-trace	4	6	8	4	9	14	9	7	2	5
	-mild	8	7	12	4	7	6	14	4	13	9
	-moderate	6	0	0	1	0	0	3	0	5	1
	-severe	3	0	1	0	0	0	0	0	0	0
Liver		(43)	(17)	(44)	(16)	(32)	(28)	(37)	(23)	(37)	(23)
Within normal limits		21	4	16	4	12	7	13	5	15	4
Cyst, biliary,		0	0	0	0	1	0	1	2	2	0
	-trace	0	0	0	0	0	0	0	0	1	0
	-mild	0	0	0	0	1	0	1	2	1	0
Hemangiosarcoma		1	0	0	0	0	0	2	0	0	0
Hemorrhage, mild		0	0	0	0	0	1	0	0	0	0
Inflammation, chronic,		0	0	3	0	2	0	5	2	0	0
	-trace	0	0	3	0	1	0	3	1	0	0
	-mild	0	0	0	0	1	0	2	1	0	0
Necrosis,		3	0	3	1	1	1	3	1	6	0
	-trace	0	0	1	0	0	0	0	0	0	0
	-mild	1	0	2	1	1	1	3	1	2	0
	-moderate	1	0	0	0	0	0	0	0	4	0
	-severe	1	0	0	0	0	0	0	0	0	0
Vacuolar change,		7	1	8	3	5	7	3	0	4	4
	-trace	2	1	2	1	0	4	3	0	2	2
	-mild	4	0	5	2	5	3	0	0	2	2
	-moderate	1	0	1	0	0	0	0	0	0	0
Adhesion, mild		0	0	1	0	0	0	0	0	0	0
Altered foci, basophilic,		2	0	0	2	1	1	2	3	1	0
	-trace	1	0	0	0	0	0	1	1	0	0
	-mild	1	0	0	2	1	1	1	2	1	0
Altered foci, clear cell,		1	1	0	2	0	1	0	2	0	3
	-trace	0	0	0	0	0	0	0	0	0	1
	-mild	1	1	0	2	0	1	0	2	0	2
Altered foci, eosinophilic,		0	0	0	0	1	1	4	0	1	1
	-trace	0	0	0	0	0	0	2	0	0	0
	-mild	0	0	0	0	1	1	2	0	1	1
Cholangioma		0	1	0	0	0	0	0	0	0	0
Congestion,		7	12	9	6	10	18	14	13	8	14
	-trace	0	4	1	1	0	3	3	7	0	6
	-mild	7	8	8	5	10	15	10	6	6	8
	-moderate	0	0	0	0	0	0	1	0	0	0
Fibrosis,		1	0	1	0	0	0	0	1	0	1
	-trace	0	0	0	0	0	0	0	0	0	1
	-mild	1	0	1	0	0	0	0	1	0	0
Adenoma, hepatocellular		0	0	0	0	0	0	1	1	0	2
Carcinoma, hepatocellular		0	0	0	0	0	1	0	0	1	0
Sarcoma, histiocytic		0	0	1	1	2	0	0	0	2	0
Hyperplasia, bile duct,		0	3	6	1	2	4	2	2	2	3
	-trace	0	3	5	0	1	4	1	2	1	3
	-mild	0	0	1	1	1	0	1	0	1	0
Leukemia, mononuclear cell		1	0	2	0	1	0	0	0	0	1
Mesothelioma, malignant		0	0	0	0	0	0	1	0	0	0
Metastatic tumor present		0	0	1	0	0	0	0	0	0	0
Spongiosis hepatis,		0	1	2	2	0	0	2	1	1	0
	-trace	0	1	2	0	0	0	0	0	1	0
	-mild	0	0	0	2	0	0	2	1	0	0
Telangiectasia,		2	0	0	2	0	0	0	1	0	0
	-trace	1	0	0	2	0	0	0	0	0	0
	-mild	1	0	0	0	0	0	0	1	0	0
Hematopoiesis, extramedullary,		1	0	0	0	0	0	1	0	2	0
	-trace	1	0	0	0	0	0	1	0	0	0
	-mild	0	0	0	0	0	0	0	0	2	0

555-023

CODE: () = NUMBER OF ANIMALS EXAMINED

+: Data excerpted from the report, p. (MRID No. 43514203).

Table 4 Cont.

Incidence of Microscopic Observations
Day 0 through Terminal Sacrifice: Rat
Female

TISSUE OBSERVATION		0 mg/kg/day (Control 1) DOS	SAC	30 mg/kg/day DOS	SAC	100 mg/kg/day DOS	SAC	400 mg/kg/day DOS	SAC	0 mg/kg/day (Control 2) DOS	SAC
Mammary Region		(43)	(17)	(38)	(13)	(23)	(14)	(37)	(23)	(37)	(23)
Within normal limits		13	1	0	0	0	0	12	3	5	3
Necrosis, moderate		0	0	0	0	0	0	1	0	0	0
Abscess, moderate		0	1	2	0	0	0	0	0	0	0
Adenocarcinoma		7	0	3	1	5	0	4	2	9	1
Adenoma		1	3	13	5	8	4	7	2	3	7
Fibroadenoma		22	11	25	8	12	12	10	13	17	14
Fibroma		0	0	0	1	2	0	0	0	0	0
Galactocele,		16	10	15	5	8	1	18	10	19	12
	-trace	1	1	0	0	0	0	3	2	0	1
	-mild	11	8	4	1	4	0	12	8	11	11
	-moderate	4	1	11	4	5	1	3	0	8	0
Ovary		(43)	(17)	(6)	(1)	(5)	(5)	(37)	(23)	(37)	(23)
Within normal limits		36	7	0	0	0	0	31	18	29	15
Cyst,		4	10	5	1	4	5	6	5	8	6
	-trace	2	1	0	0	0	0	1	0	0	0
	-mild	1	7	2	1	3	4	2	4	5	5
	-moderate	1	2	3	0	1	1	3	1	2	0
	-severe	0	0	0	0	0	0	0	0	1	1
Congestion, mild		1	0	0	0	0	0	0	0	0	0
Granulosa cell tumor, benign		1	0	1	0	0	0	0	0	0	0
Granulosa cell tumor, malignant		1	0	0	0	0	0	0	0	0	0
Leukemia, mononuclear cell		0	0	0	0	1	0	0	0	0	1
Thecoma		0	0	0	0	0	1	0	1	0	1
Uterus		(43)	(17)	(11)	(12)	(6)	(14)	(37)	(23)	(37)	(23)
Within normal limits		35	6	0	0	0	1	27	9	24	16
Cyst,		4	8	2	9	2	9	7	11	9	6
	-trace	0	0	1	0	0	0	2	1	0	1
	-mild	4	6	1	5	0	7	5	10	8	5
	-moderate	0	2	0	4	2	2	0	0	1	0
Dilatation, tubular,		4	10	5	3	3	2	3	1	4	2
	-trace	0	0	0	1	1	1	0	0	0	0
	-mild	3	8	5	0	1	1	2	1	3	0
	-moderate	1	2	0	2	1	0	1	0	1	2
Hemorrhage, mild		0	0	0	0	0	0	0	0	1	0
Hyperplasia, cystic, mild		0	2	0	0	0	0	0	0	1	0
Leiomyosarcoma		0	0	1	0	0	0	0	0	0	1
Polyp		3	0	4	2	1	3	1	5	1	1
Uterus, Cervix		(43)	(17)	(1)	(0)	(0)	(0)	(37)	(23)	(37)	(23)
Within normal limits		43	17	0	0	0	0	37	23	35	23
Cyst, mild		0	0	0	0	0	0	0	0	1	0
Cyst, epidermal, severe		0	0	0	0	0	0	0	0	1	0
Sarcoma, endometrial		0	0	1	0	0	0	0	0	0	0
Vagina		(43)	(17)	(2)	(0)	(0)	(0)	(37)	(23)	(37)	(23)
Within normal limits		41	17	0	0	0	0	36	22	37	22
Hemorrhage, moderate		0	0	1	0	0	0	0	0	0	0
Hyperkeratosis, trace		0	0	0	0	0	0	0	0	0	1
Inflammation, acute, mild		0	0	0	0	0	0	1	0	0	0
Cyst, epidermal,		1	0	0	0	0	0	0	1	0	0
	-trace	0	0	0	0	0	0	0	1	0	0
	-mild	1	0	0	0	0	0	0	0	0	0
Polyp		1	0	1	0	0	0	0	0	0	0

555-023 CODE: () = NUMBER OF ANIMALS EXAMINED

+: Data excerpted from the report, p. (MRID No. 43514203).

Appendix A+

Statistics

Body weights, food consumption, clinical pathology laboratory values and organ weights (absolute and relative to body and brain weights) were analyzed using one-way analysis of variance (ANOVA). If the ANOVA was not significant, no further test was performed; otherwise, a Bartlett's test for homogeneity of variance was performed as described by Steel and Torrie[1] followed by the appropriate pairwise comparisons. If the Bartlett's test was not significant, a Dunnett's[2] t-test was used for the pairwise comparisons; otherwise, the Welch[3] t-test with a Bonferroni[4] correction was used. When non-parametric statistical procedures were required, the rank transformation methods described by Conover and Iman[5] were used. All pairwise comparisons consisted of comparing treatment groups to the control groups. Statistical tests were conducted at the 0.05 and 0.01 levels of significance. Tumor incidence data were analyzed as described by Huff[6]. Statistical procedures included the life table test, Hoel-Walburg "incidental tumor" test, Fisher's exact test and Cochran-Armitage trend test. Microscopic lesions in tissues from animals with the low- and mid-dose groups that were examined only as a result of a gross finding were not analyzed statistically.

Two untreated control groups were included in this study. These groups were treated as independent entities for all activities performed during the study, such as assignment of animals to groups, placement of cages on racks, collection of in-life data and order of blood collection and sacrifice. The purpose of including two control groups in this study was to collect data which would provide some information regarding the range of normal or control values for the parameters evaluated in this study. These data were used as an aid in the identification of false positive statistical citations as well as confirmation of true treatment-related effects. Based on the independent manner in which the animals were handled and the data were collected, it was not considered appropriate to combine the data from the two control groups for the purposes of comparing the combined control data to those from the treated groups.

+: Information excerpted from the report; p. 20 & 21 (MRID No. 43514203).

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Reviewer: Whang Phang, Ph.D. 8/1/95
Tox. Branch II (7509C)
Secondary Reviewer: James Rowe, Ph.D. 8/1/95
Tox. Branch II (7509C)

DATA EVALUATION REPORT

Study Type: 8-Week dietary dose-range finding study in dogs

Chemical: DEET (N,N-diethyl-m-toluamide)

Caswell No.	346	**DP Barcode Code:**	D211262
MRID No.	43514202	**PC Code:**	080301
EPA ID No.	N80301-051147	**Submission No.:**	S480555

Sponsor: DEET Joint Venture/Chemical Specialties Manufacturers Association

Testing Facility: International Research and Development Corp.
500 N. Main
Mattawan, Michigan 49071

Citation: Goldenthal, E.I. (1994) Evaluation of DEET in an eight week dietary toxicity study in dogs. International Research and Development Corp.; Study No. 555-020. January 3, 1995. Submitted to EPA by CSMA. EPA MRID No. 43514202

Conclusion: In an 8-week dose-range finding study, groups of beagle dogs (2/sex/dose) received DEET in the diet at concentrations of 300, 1000, 3000, or 6000/4500/3000 ppm[1] (8.4, 28.6, 93.3, or 19.5 mg/kg for males and 9.7, 30.6, 91.8, or 11.5 mg/kg for females). The control animals received basal diet.

Under the conditions of this study, DEET did not produce any toxicity at dietary concentrations of 3000 ppm or less. At a concentration of 6000/4500/3000 ppm, DEET caused food rejection which led to a decrease in body weight, thin appearance, fat depletion, organ weight decrease, and histological changed in kidneys, bone marrow, and thymus.

The reliability of the results of this study is compromised by the small no of dogs (2/sex/dose) used, and a useful NOEL and LEL could not be established.

This study is classified as **supplementary**, and does not meet the data requirements for subchronic oral toxicity study in dogs (82-1).

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[1] During the first two weeks of the study, the highest dose male and female dogs received 6000 ppm test diet, but the dogs rejected the test diet. The treatment diet was withdrawn at the end of the second week, and the animals were given the basal diet for ≈1 week. The dosage was then reduced at week 4 from 6000 ppm to 4500 ppm and at week 7 from 4500 ppm to 3000 ppm. At week 6, this dose group of dogs was again given the basal diet.

Methods and Materials

Test article: Technical DEET (98.3%) was "a mixture consisting of equal parts of four representative production runs" supplied by four manufacturers (McLaughlin Gormley King Co, Miles Lab., Virginia Chemical Co., and Morflex Chemical Co.). The test article was described as a clear liquid (Lot No. A-1-96) and assigned the ID No. IRDC 8812B at the testing laboratory. The test article was found to be stable at room temperature.

Test animals: Twelve male and 12 female purebred beagle dogs (≈ 5 months of age) were obtained from Ridglan Farms, Mt. Horeb, Wisconsin. during the 4 week acclimation periods all dogs received immunization and a physical examination.

Study Design

1. Animal assignments: Ten male and 10 female beagle dogs were selected for this study. The body weights of males were in the range of 9.3 to 12.6 kg; females, 7.7 to 10.2 kg. The test animals were divided into 4 treatment groups and a control group based on body weights (a large dog was paired with a smaller dog of the same sex) as follows:

Dosage Levels ppm	Number of Animals	
	Males	Female
(control) 0	2	2
300	2	2
1000	2	2
3000	2	2
6000/4500/3000*	2	2

*: During the first two weeks of the study, this group of dogs received DEET at a concentration of 6000 ppm, but the test animals rejected the test diet. The test diet was withdrawn, and the animals were given the control diet for ≈1 week. The dosage was continuously reduced at week 4 from 6000 to 4500 and at week 7 from 4500 to 3000. At week 6, the test animals were offered basal diet again.

2. Test article preparation and administration: The test diet was prepared weekly by appropriately diluting the premix with the diet. The premix was prepared by mixing DEET with the ground diet in a Hobart Blender. The prepared diet was stored at room temperature. Samples of prepared diet (100 gm/sample) were taken for analysis of homogeneity, stability, and verification of concentrations.

3. Physical examinations: Physical examinations were conducted on each dog at pretest and at termination. In addition stool floatation tests were conducted on all dogs.

4. Observations: The test animals were observed for any clinical signs of toxicity, moribundity, and mortality twice daily throughout the study. Detailed clinical observations were also conducted at least once weekly.

5. Body weight and food consumption: Individual body weight measurements were determined at pretest and weekly during the study. Individual food consumption and compound intake were determined weekly throughout the study period.

6. Hematology and biochemical analyses: Blood samples were collected from the test animals following an overnight fast. Hematology and biochemical analyses were conducted using the blood samples collected prior to the initiation of the study and at the termination of the study.

Hematology: The following hematological parameters were measured:

erythrocyte count	hemoglobin
leukocyte count	differential leukocyte count
hematocrit	platelet
reticulocyte count	Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)	Mean corpuscular hemoglobin concentration (MCHC)

Clinical chemistry: The following biochemistry parameters were determined:

sodium	potassium
chloride	calcium
phosphorus	total bilirubin
aspartate aminotransferase (AST) (SGOT)	alanine aminotransferase (ALT) (SGPT)
urea nitrogen	creatinine
total protein	albumin
globulin	glucose
alkaline phosphatase	creatine phosphokinase (CPK)
cholesterol	

6. Pathology: At the end of 8 weeks, all animals were weighed and sacrificed with sodium pentobarbital.

a. Necropsy: A thorough postmortem examination was conducted on each animal. The abdominal, thoracic, and cranial cavities were examined for abnormalities.

b. Organ weights: The following organs were removed, trimmed free of fat, and weighed:

adrenals	liver
brain	ovaries
kidneys	testis with epididymis
heart	pituitary
thyroid/parathyroid	spleen

The following organs were removed and placed in the phosphate-buffered neutral formalin.

adrenal	kidney (2)
aorta	liver
bone (femur & rib)	lung with bronchi
bone marrow & smears	lymph ones (tracheobronchial & mesenteric)
brain	eye with optic nerve
gallbladder	pancreas
mammary gland	pituitary
GI tract:	prostate
esophagus	salivary gland
stomach	sciatic nerve
duodenum	skeletal muscle (thigh)
jejunum	skin
ileum	spinal cord
cecum	spleen
colon	sternum
rectum	thymus
ovary	thyroid/parathyroid
testes with epididymis	trachea
heart	uterus
urinary bladder	
gross lesions	

d. Histopathology examination:

A full complement of organs and tissues consisted of the following:

adrenals	lung with bronchi
bone & bone marrow	liver
kidneys	lymph nodes (tracheobronchial and mesenteric)
ovary	pancreas
testis with epididymis	pituitary
heart	spleen
spinal cord (entire)	thyroid/parathyroid
thymic region	
gross lesions	

A grading system (trace, mild, moderate, and severe) was used to define any gradable lesions for comparison purposes.

7. Statistics: Statistical analysis was not conducted due to the small number (2/group) in each dose group.

8. Quality assurance: A statement of no data confidentiality claim, a statement of compliance, and a quality assurance statement were signed and included in the report.

Results

1. Diet analysis: Stability analysis was not conducted for this study. However, based on the data from a previous study (MRID No. 43514203; IRDC Project No. 555-023), DEET was found to be stable at room temperature for 14 days. The samples of the test diet were found to contain DEET at levels between 88% to 108% of the targeted dietary concentrations. DEET was reported to be uniformally mixed in the diet based on results of a previous study (MRID No. 43514203) employing mixing procedures identical to those used in this study.

2. Clinical observation: The clinical observation data showed that 6000/4600/3000 ppm males and females were thin and had decreased activity (Table 1; p. 9 & 10). Other compound-related clinical signs were not found.

2. Survival rates: No deaths occurred during the study.

3. Physical examination: The physical examination did not revealed a compound-related effect in 3000 ppm or lower concentrations of the treated dogs. At a concentration level of 6000/4600/3000 ppm groups, all the test animals were thin.

4. Body weights: The mean body weight values were excerpted from the report and presented in Table 2. In both males and females at concentrations of 3000 ppm or lower, the body weights measured at 8 weeks were comparable between the treated and the controls. The body weights of 6000/4500/3000 ppm males and females at 8 weeks were less than those at pretest by as much as 9% and 30%, respectively (Table 2). The test animals in other dose groups all had gained some weights.

5. Food consumption: The food consumption data were comparable among the controls, 300, 1000, and 3000 ppm males and females. Both males and females of 6000/4500/3000 ppm groups had reduced food intake, and that in female dogs was more marked (Table 3). The individual animal data on 6000/4500/3000 ppm male and female dogs indicated that the test animals found the test diet at this dose level to be unpalatable and rejected the high dose test diet. As soon as the test diet was withdrawn and switched to the control diet at Weeks 3 and 6,

the food consumption went up dramatically (Table 4; p. 11). Despite a continuous lowering of the concentration of DEET in the high dose diet, the test animals continue to consume much less food.

Table 2+. Mean Body Weights (kg)

Dose Levels (ppm)	Males		Females	
	Pretest 3	8-weeks	Pretest	8-Weeks
0 (Cont.)	11.0	12.2 (11)	9.0	9.8 (9)
300	11.3	12.4 (10)	9.1	10.2 (12)
1000	11.3	12.4 (10)	9.2	9.4 (2)
3000	11.1	12.8 (15)	9.1	10.2 (12)
6000/4500/ 3000	11.0	8.9 (-19)	9.0	6.3 (-30)

+: Data excerpted from the individual animal data of the report; p. 108-117 (MRID No.43514202).
( ): % difference from the pretest.
Cont: Control
Pretest 3: The values represent those measured at the third pre-test determination.

Table 3+: Average Food Consumption During the Study[a]

Dose Levels (ppm)	Males (g/dog/day)	Females (g/dog/day)
0 (Control)	355	294
300	343 (97)	319 (109)
1000	356 (100.3)	290 (99)
3000	393 (111)	305 (104)
6000/4500/3000	226 (64)	173 (59)

+: Data excerpted from the report; p.119-128 (MRID No.43514202).
a: These values were calculated from the mean weekly food consumption data.
(): % of the control.

6. Compound Intake: The average daily dose of test chemical was calculated and summarized in Table 4. The results showed that the daily dose of DEET received by female and male dogs at each dose level was comparable. The daily dose for 6000/4500/ 3000 ppm males and females was substantially less than 1000 and 3000 ppm dogs.

Table 4+. Average Compound Intake in DEET Treated Dogs.

Dose level (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
300	8.4	9.7
1000	28.6	30.6
3000	93.3	91.8
6000/4500/3000	19.5	11.5

+: Data calculated from the results in the report; p.130-139 (MRID No.43514202).

7. Hematology: The hematological data did not demonstrate a compound-related change.

8. Clinical chemistry: There were no compound-related changes in the clinical chemistry parameters in any dose groups.

9. Macroscopic: The gross examination data showed that one female dog in 6000/4500/3000 ppm group had signs of fat depletion. Other findings were comparable between the treated and the control dogs (Table 5; p. 12).

10. Organ weights: The organ weight data were excerpted from the report and presented in Tables 6a & 6b (p.13). In 6000/4500/3000 ppm males and females, there was a decrease in essentially all organ weights relative to those of the controls. This finding was associated with a marked decrease in food intake and reduced body weights.

10. Histopathology: In 6000/4500/3000 ppm groups, there was an increase in the incidence of hypocellularity in bone marrow of the ribs in 2/2 males and 2/2 females; 1/2 males and 2/2 females had cytoplasmic vacuolization of tubules in the cortex of the kidneys; thymic atrophy and hemorrhage were seen in 1/2 males and 2/2 females. No histological changes were seen in animals of 3000 ppm or lower dose groups.

Since the dosage of DEET received by the 6000/4500/3000 ppm dogs (11.5 mg/kg for males and 11.5 for females) was substantially less than those received by 1000 or 3000 ppm dogs (≈30 mg/kg and ≈92 mg/kg, respectively) and no toxicity was found in 1000 and 3000 ppm dogs, the histological changes in the bone marrow, kidneys, and thymus could be attributed to the marked reduction of food intake in 6000/4500/3000 ppm dogs.

Discussion

Groups of beagle dogs (2/sex/dose) received DEET in the diet at concentrations of 300, 1000, 3000, or 6000/4500/3000 ppm (8.4, 28.6, 93.3, or 19.5 mg/kg for males and 9.7, 30.6, 91.8, or 11.5 mg/kg for females). The control animals received basal diet. During the first two weeks of the study, the 6000 ppm male and female dogs rejected the test diet. The treatment diet was withdrawn at the end of the second week, and the animals were given the basal diet for the 3rd week. The dosage was continuously reduced at week 4 from 6000 ppm to 4500 ppm and at week 7 from 4500 ppm to 3000 ppm.

Under the conditions of this study, DEET did not produce any toxicity at dietary concentrations of 3000 ppm or less. At concentrations of 6000/4500/3000 ppm, DEET caused food rejection which led to a decrease in body weight, thin appearance, fat depletion, organ weight decrease, and histological changed in kidneys, bone marrow, and thymus.

The reliability of the results of this study is compromised by the small number of dogs (2/sex/dose) used, and a useful NOEL and LEL could not be established.

This study is classified as **supplementary**, and do not meet the data requirements for subchronic oral toxicity study in dogs (82-1).

Table — Summary of Clinical Findings
MALES

Observation	Interval: 1 - 8 Week 0 ppm (2)	300 ppm (2)	1,000 ppm (2)	3,000 ppm (2)	Interval: 1 - 8 Week 6,000/4,000/3,000 ppm (2)
APPEARANCE AND CONDITION					
No visible abnormalities for entire interval	0	0	0	0	0
Ear abnormality	0	0	1 [50.0]	0	0
Thin	0	0	0	0	2 [100.0]
Abnormal material below cage	0	1 [50.0]	0	0	1 [50.0]
Subcutaneous mass	0	0	0	1 [50.0]	0
Portion external ear missing	1 [50.0]	0	0	1 [50.0]	0
BEHAVIOR/ACTIVITY					
Decreased activity	0	1 [50.0]	0	0	2 [100.0]
EXCRETION					
Diarrhea	1 [50.0]	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]
Decreased defecation	1 [50.0]	2 [100.0]	1 [50.0]	2 [100.0]	2 [100.0]
Food-like emesis	1 [50.0]	2 [100.0]	2 [100.0]	1 [50.0]	0
Frothy emesis	0	2 [100.0]	1 [50.0]	0	2 [100.0]
Mucoid diarrhea	0	1 [50.0]	0	0	1 [50.0]
Soft stool	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]
BODY SURFACE					
Abrasion	0	0	0	0	1 [50.0]
Alopecia	0	0	1 [50.0]	0	0
Dermatitis	0	1 [50.0]	0	0	0
Growth	0	1 [50.0]	0	0	0
Red material	0	0	0	0	1 [50.0]
Wart-like growth	0	2 [100.0]	0	0	0
Erythema	0	0	0	0	1 [50.0]
Lacerations	1 [50.0]	0	0	1 [50.0]	0
ORAL/NASAL					
Papillomas	0	0	1 [50.0]	1 [50.0]	0
EYES					
Excessive lacrimation	0	1 [50.0]	0	0	0
Ocular discharge	0	1 [50.0]	0	0	0
Injection of sclera	2 [100.0]	2 [100.0]	1 [50.0]	0	0
Relaxed nictitating membrane	1 [50.0]	1 [50.0]	0	1 [50.0]	0

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( ) = Number of animals observed at start of interval
[ ] = Percent of animals with observation during interval

+: Data excerpted from the report; p. 38-39 (MRID No. 43514202).

TABLE CONT.

Summary of Clinical Findings
FEMALES

Observation	Interval: 1 - 8 Week 0 ppm (2)	300 ppm (2)	1,000 ppm (2)	3,000 ppm (2)	Interval: 1 - 8 Week 6,000/4,500/3,000 ppm (2)
APPEARANCE AND CONDITION					
No visible abnormalities for entire interval	0	0	0	0	0
Thin	0	0	0	0	2 [100.0]
Umbilical hernia	0	0	1 [50.0]	0	0
BEHAVIOR/ACTIVITY					
Decreased activity	0	0	0	0	2 [100.0]
EXCRETION					
Diarrhea	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]
Decreased defecation	1 [50.0]	0	1 [50.0]	2 [100.0]	2 [100.0]
Discolored urine	0	0	0	0	1 [50.0]
Emesis	0	1 [50.0]	0	0	0
Food-like emesis	1 [50.0]	1 [50.0]	2 [100.0]	2 [100.0]	0
Frothy emesis	1 [50.0]	0	0	2 [100.0]	0
Mucoid diarrhea	0	0	0	1 [50.0]	1 [50.0]
Soft stool	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]	2 [100.0]
Discolored feces	0	0	0	0	1 [50.0]
BODY SURFACE					
Cyst	0	0	1 [50.0]	0	0
Dermatitis	1 [50.0]	0	0	0	0
ORAL/NASAL					
Dry nose	1 [50.0]	0	0	0	0
Papillomas	0	1 [50.0]	0	0	0
EYES					
Injection of sclera	1 [50.0]	2 [100.0]	2 [100.0]	1 [50.0]	1 [50.0]
Relaxed nictitating membrane	1 [50.0]	1 [50.0]	0	0	0

555-020

( ) = Number of animals observed at start of interval
[ ] = Percent of animals with observation during interval

+: Data excerpted from the report; p. 40 - 41 (MRID No. 43514202).

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TABLE 4.+

Individual Food Consumption, Grams/day

GROUP, ANIMAL NO.	SEX	STUDY WEEK 1	2	3a	3b	4	5	6c	7	8
6,000/4,500/3,000 ppm										
2217	M	20	12	733*	54*	60	4	692	12	24*
2220	M	28d	4	661*	17*	26	2	723	24	532
MEAN		20	8	697	36	43	3	708	18	278
6,000/4,500/3,000 ppm										
2238	F	5	28	636*	16*	4	12	682	16	16*
2240	F	21	8	563*	7*	18	5	601	40	86
MEAN		13	18	600	12	11	9	642	28	51

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* - Variation in the number of days used for calculation of food consumption
a - 6 day food consumption, animals did not receive test substance for this interval
b - 1 day food consumption, animals received test substance for this interval
c - Animals did not receive test substance for this interval

+: Data excerpted from the report; p. 127-128 (MRID No. 43514202).

Table 5+

INCIDENCE OF MACROSCOPIC OBSERVATIONS
Terminal Sacrifice - Males and Females

SITE - Observation		0 ppm (Control) M	0 ppm (Control) F	300 ppm M	300 ppm F	1,000 ppm M	1,000 ppm F	3,000 ppm M	3,000 ppm F	6,000/4,500/3,000 ppm M	6,000/4,500/3,000 ppm F
NUMBER OF ANIMALS EXAMINED		2	2	2	2	2	2	2	2	2	2
NUMBER WITHIN NORMAL LIMITS		0	1	0	1	0	0	0	1	1	0
BODY											
- Depletion of body fat, moderate											1
EYE											
- Sclera, discolored, red,	- trace			1			1				
	- mild	1		1	1		1		1		
LIVER											
- Focus, tan, trace					1						
LUNG											
- Discolored, red, mild				1							
- Foci, tan,	- trace	1									
	- mild				1						
- Foci, red, trace		1									
LYMPH NODE											
- Popliteal, enlarged,	- trace							1			
	- mild	1									
LYMPH NODE, MANDIBULAR											
- Enlarged, NOS				1							
ORAL TISSUES											
- Buccal mucosa/mucosa, nodule		1		1							
PITUITARY											
- Cyst, mild									1		
SKIN, EAR											
- Portion missing, no grade		1						1			
- Thickened, mild						1					
SKIN, LIP											
- Nodule			1	1		1		1			
SKIN, NOSE											
- Scabbed area, mild										1	
SKIN/SUBCUTIS											
- Raised area, mild				1							
- Thickened, mild							1				
SPLEEN											
- Focus, white, mild					1						1
THYMUS											
- Discolored, red, moderate											1

555-020 +: Data excerpted from the report; p. 58+59 (MRID No. 43514202).

Table 6a+. Organ Weights in the Control and DEET Treated Dogs.

Parameters Measured	0 ppm	300 ppm	1000 ppm	3000 ppm	6000/4500/ 3000 ppm
	Males				
Body weights, kg	12.2	12.4	12.2	12.7	8.8
Brain, g	84.6	77.95	80.5	82.7	76.8
Adrenal(L), kg	0.74	0.53	0.64	0.59	0.51
Heart, g	100.7	87.9	92.9	94.2	69.1
Kidney (L), g	28.9	26.2	25.5	27.3	24.7
Liver/gall bladder, g	a	320.9	330.2	323.9	286.9
Pituitary, g	74	65	67	78	67
Spleen, g	69.4	71.5	40.3	91.0	27.0
Testis/epididymis (L), g	11.4	8.1	11.8	10.5	6.8
Thyroid/parathyroid (L), g	0.89	0.76	0.88	0.75	0.85

+: Data excerpted from the report; p. 60-69 (MRID No. 43514201).

a: No data was given in the report.

Table 6b+. Organ Weights in the Control and DEET Treated Dogs.

Parameter Measured	0 ppm	300 ppm	1000 ppm	3000 ppm	6000/4500/3000 pm
	Females				
Body weights, kg	9.7	10.1	9.4	10.1	6.3
Brain, g	73.3	71.1	73.5	72.4	68.7
Adrenal(L), kg	0.65	0.70	0.61	0.54	0.54
Heart, g	78.1	77.2	76.4	71.4	51.2
Kidney (L), g	21.2	23.7	20.7	21.1	18.6
Liver/gallbladder, g	277.7	260.8	238.1	271.8	182.0
Pituitary, g	54	60	70	95	52
Spleen, g	29.4	52.3	35.5	32.5	29.2
Ovary (L), g	0.45	0.57	0.50	0.47	0.35
Thyroid/parathyroid (L), g	0.69	0.99	0.62	0.67	0.52

+: Data excerpted from the report; p. 70-81 (MRID No. 43514201).

Table 7+.

Incidence of Microscopic Observations
Terminal Sacrifice: Dogs
Male

TISSUE OBSERVATION		0 ppm (Control)	300 ppm	1,000 ppm	3,000 ppm	6,000/4,500 /3,000 ppm
Adrenal, Cortex		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Adrenal, Medulla		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Bone Marrow, Rib		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	0
Hypocellular,		0	0	0	0	2
	-trace	0	0	0	0	1
	-moderate	0	0	0	0	1
Bone, Rib		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Epididymis		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	1	2
Periarteritis, moderate		0	0	0	1	0
Eye		(1)	(2)	(0)	(0)	(0)
Within normal limits		1	2	0	0	0
Heart		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	1
Hyperplasia, epicardial, papillary, mild		0	0	0	0	1
Kidney		(2)	(2)	(2)	(2)	(2)
Mineralization, trace		2	2	2	2	2
Vacuolar change, moderate		0	0	0	0	1
Liver		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	1	2	2
Inflammation, chronic, trace		0	0	1	0	0
Lung		(2)	(2)	(2)	(2)	(2)
Within normal limits		1	1	2	1	2
Hemorrhage, mild		0	1	0	0	0
Interstitial pneumonia,		1	1	0	1	0
	-trace	1	0	0	0	0
	-mild	0	1	0	1	0
Lymph Node, Mandibular		(0)	(1)	(0)	(0)	(0)
Lymphoid hyperplasia, mild		0	1	0	0	0
Lymph Node, Mesenteric		(2)	(2)	(2)	(2)	(2)
Within normal limits		1	1	2	2	2
Erythrophagocytosis, mild		1	1	0	0	0
Lymph Node, Popliteal		(1)	(0)	(0)	(1)	(0)
Within normal limits		1	0	0	0	0
Lymphoid hyperplasia, mild		0	0	0	1	0
Lymph Node, Tracheobronchial		(2)	(2)	(2)	(2)	(2)
Within normal limits		1	1	2	2	2
Erythrophagocytosis,		1	1	0	0	0
	-trace	0	1	0	0	0
	-mild	1	0	0	0	0
Oral Tissues		(1)	(2)	(1)	(1)	(0)
Papilloma		1	2	1	1	0
Pancreas		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Parathyroid		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	1
Cyst, trace		0	0	0	0	1
Pituitary		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	1
Cyst, mild		0	0	0	0	1
Skin		(0)	(2)	(0)	(0)	(0)
Inflammation, chronic, mild		0	1	0	0	0

568-020 CODE: () = [redacted]

+: Data excerpted from the report; p. 82-85 ([redacted]).

Incidence of Microscopic Observations
Terminal Sacrifice: Dogs
Male

Table Cont.

TISSUE OBSERVATION	0 ppm (Control)	300 ppm	1,000 ppm	3,000 ppm	6,000/4,500 /3,000 ppm
Thymus	(2)	(2)	(2)	(2)	(2)
Within normal limits	2	2	2	2	1
Atrophy, moderate	0	0	0	0	1
Hemorrhage, moderate	0	0	0	0	1
Thyroid	(2)	(2)	(2)	(2)	(2)
Within normal limits	1	1	1	2	1
C-cell hyperplasia, mild	1	1	1	0	1
Skin, Ear	(0)	(0)	(1)	(0)	(0)
Inflammation, chronic, mild	0	0	1	0	0
Skin, Nose	(0)	(0)	(0)	(0)	(1)
Inflammation, chronic, mild	0	0	0	0	1
Spleen	(2)	(2)	(2)	(2)	(2)
Within normal limits	2	2	2	2	2
Testis	(2)	(2)	(2)	(2)	(2)
Within normal limits	2	0	2	2	2
Atrophy,	0	2	0	0	0
-mild	0	1	0	0	0
-moderate	0	1	0	0	0

555-020 CODE: () = NUMBER OF ANIMALS EXAMINED

011625

Incidence of Microscopic Observations
Terminal Sacrifice: Dogs
Female

Table 7[4] CONT.

TISSUE OBSERVATION		0 ppm (Control)	300 ppm	1,000 ppm	3,000 ppm	6,000/4,500 /3,000 ppm
Adrenal, Cortex		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Adrenal, Medulla		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Bone Marrow, Rib		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	0
Hypocellular,		0	0	0	0	2
	-mild	0	0	0	0	1
	-moderate	0	0	0	0	1
Bone, Rib		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Eye		(0)	(1)	(2)	(1)	(0)
Within normal limits		0	1	2	1	0
Heart		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	1	2	2	2
Periarteritis, trace		0	1	0	0	0
Kidney		(2)	(2)	(2)	(2)	(2)
Mineralization,		2	2	2	2	2
	-trace	2	2	1	2	1
	-mild	0	0	1	0	1
Vacuolar change,		0	0	0	0	2
	-mild	0	0	0	0	1
	-moderate	0	0	0	0	1
Liver		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	1	1	2	2
Inflammation, chronic, trace		0	1	1	0	0
Lung		(2)	(2)	(2)	(2)	(2)
Within normal limits		1	1	1	2	2
Interstitial pneumonia, trace		1	0	1	0	0
Nematodiasis, trace		0	1	0	0	0
Lymph Node, Mesenteric		(2)	(2)	(2)	(2)	(2)
Within normal limits		1	2	2	2	2
Erythrophagocytosis, mild		1	0	0	0	0
Lymph Node, Tracheobronchial		(1)	(2)	(2)	(2)	(2)
Within normal limits		1	2	2	2	2
Oral Tissues		(1)	(0)	(0)	(0)	(0)
Papilloma		1	0	0	0	0
Ovary		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Pancreas		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Parathyroid		(2)	(2)	(2)	(2)	(2)
Within normal limits		1	2	2	2	1
Cyst,		1	0	0	0	1
	-trace	1	0	0	0	0
	-mild	0	0	0	0	1
Pituitary		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	1	2	1	2
Cyst,		0	1	0	1	0
	-trace	0	1	0	0	0
	-moderate	0	0	0	1	0
Skin		(0)	(0)	(1)	(0)	(0)
Inflammation, chronic, trace		0	0	1	0	0
Spleen		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2
Thymus		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	0
Atrophy,		0	0	0	0	2
	-mild	0	0	0	0	1
	-moderate	0	0	0	0	1
Hemorrhage,		0	0	0	0	2
	-trace	0	0	0	0	1
	-mild	0	0	0	0	1
Thyroid		(2)	(2)	(2)	(2)	(2)
Within normal limits		2	2	2	2	2

 CODE: ( ) = NUMBER OF ANIMALS EXAMINED +: Data excerpted from the report; p. 86-88 (MRID No. 43514202).

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Reviewer: Whang Phang, Ph.D. 6/16/95
Tox. Branch II (7509C)

Secondary Reviewer: James Rowe, Ph.D. James N. Rowe 6/16/95
Tox. Branch II (7509C)

DATA EVALUATION REPORT

Study Type: 8-Week feeding dose-range finding study in dogs (Oral gelatin capsule)

Chemical: DEET (N, N-diethyl-m-toluamide)

Caswell No.	346	**DP Barcode Code:**	D211262
MRID No.	43514201	**PC Code:**	080301
EPA ID No.	N80301-051147	**Submission No.:**	S480555

Sponsor: DEET Joint Venture/Chemical Specialties Manufacturers Association

Testing Facility: International Research and Development Corp.
500 N. Main
Mattawan, Michigan 49071

Citation: Goldenthal, E.I. (1994) Evaluation of DEET in an eight week oral gelatin capsule toxicity study in dogs. International Research and Development Corp.; Study No. 555-027. January 3, 1995. Submitted to EPA by CSMA. EPA MRID No. 43514201

Conclusion: In a 8-week dose-range finding study, groups of beagle dogs (2/sex/dose) received DEET in a gelatin capsule at dose levels of 50, 100, 200, or 400 mg/kg/day. The control animals received white mineral oil in gelatin capsule. The following results were obtained:

1. Clinical observation data showed a significant increase in ptyalism in 100 mg/kg or above males and females and an increase in abnormal head movements in 400 mg/kg males.

2. A decrease in body weight gains was found in 400 mg/kg males and females, and that in female dogs was more marked.

3. Food consumption was substantially reduced in 400 mg/kg females.

4. There was a decrease in cholesterol level in 400 mg/kg male dogs.

5. A decrease in testis/epididymis weight was found in 400 mg/kg males. However, both gross examination and histopathology did not indicate any changes in the testis or any other organs.

The reliability of the results of this study is compromised by the small number of dogs (2/sex/dose) used, and a useful NOEL and LEL could not be established.

Based on the above results, the registrant selected 400 mg/kg as the highest dose and 30 and 100 mg/kg as low and mid dose, respectively, for a chronic toxicity study in dogs. The selected doses for the chronic toxicity appeared to be adequate.

This study is classified as **supplementary**, and does not meet the data requirements for a subchronic oral toxicity study in dogs (82-1).

Methods and Materials

Test article: Technical DEET (98.3%) was "a mixture consisting of equal parts of four representative production runs" supplied by four manufacturers (McLaughlin Gormley King Co, Miles Lab., Virginia Chemical Co., and Morflex Chemical Co.). The test article was a pale yellow liquid (Lot No. A-1-96) and assigned the ID No. IRDC 8812B at the testing laboratory. The test article was found to be stable at room temperature.

Test animals: Twelve male and 12 female purebred beagle dogs (≈4 to 5 months of age) were obtained from Ridglan Farms, Mt. Horeb, Wisconsin.

Study Design

1. Animal assignments: Ten male and 10 female beagle dogs were selected for this study. The body weights of males were in the range of 7.1 to 10.7 kg; females, 6.5 to 9.3 kg. The test animals were divided into 4 treatment groups and a control group as follows:

Dosage Levels mg/kg	Number of Animals	
	Males	Female
(control) 0	2	2
50	2	2
100	2	2
200	2	2
400	2	2

2. Test article preparation and administration: With a glass syringe, an appropriate amount of DEET was placed into a gelatin capsule, which has a volume of approximately 7 ml. For the control group, an appropriate amount of white mineral

oil was placed into the capsule. The volume of DEET or white mineral oil placed into a capsule was based on a test animal's most recent body weight measurement.

The stability of DEET in capsule was analyzed after storing the prepared capsule for 14 days at room temperature. At the end of the 14 days, aliquots of DEET from the capsule and that from the stock solution were analyzed and compared. **The results indicated that DEET was stable in the gelatin capsule for at least 14 days.**

Each animal was dosed twice daily in equally divided doses of 25, 50, 100, and 100 mg/kg/day. The animals were dosed one hour following food (7:30 am and 1:00 pm), 7 days/week, throughout the study. The control dogs received white mineral oil in similar treatment schedules.

3. Physical examinations: Physical examinations were conducted on each dog at pretest and at termination. The examinations included auscultation of the thoracic cavity and respiratory tract and palpitation the thoracic cage and abdomen.

4. Observations: The test animals were observed for any clinical signs of toxicity, moribundity, and mortality twice daily throughout the study.

5. Body weight and food consumption: Individual body weight measurements were determined at pretest and weekly during the study. Individual food compound consumption was determined weekly throughout the study period.

6. Hematology and biochemical analyses: Blood samples were collected from the test animals following an overnight fast. Hematology and biochemical analyses were conducted using the blood samples collected prior to the initiation of the study and at the termination of the study.

Hematology: The following hematological parameters were measured:

erythrocyte count	hemoglobin
leukocyte count	differential leukocyte count
hematocrit	platelet
reticulocyte count	Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)	Mean corpuscular hemoglobin concentration (MCHC)

Clinical chemistry: The following biochemistry parameters were determined:

sodium	potassium
chloride	calcium
phosphorus	total bilirubin
aspartate aminotransferase (AST) (SGOT)	alanine aminotransferase (ALT) (SGPT)
urea nitrogen	creatinine
total protein	albumin
globulin	glucose
alkaline phosphatase	creatine phosphokinase (CPK)
cholesterol	

6. Pathology: At the end of 8 weeks, all animals were weighed and sacrificed.

a. Necropsy: A thorough postmortem examination was conducted on each animal. The abdominal, thoracic, and cranial cavities were examined for abnormalities.

b. Organ weights: The following organs were removed, trimmed free of fat, and weighed:

adrenals	liver
brain	ovaries
kidneys	testis with epididymis
heart	pituitary
thyroid/parathyroid	

The following organs were removed and placed in the phosphate-buffered neutral formalin.

adrenal	kidney (2)
aorta	liver
bone (femur & rib)	lung with bronchi
bone marrow & smears	lymph ones (tracheobronchial & mesenteric)
brain	mammary gland
eye with optic nerve	pancreas
gallbladder	pituitary
GI tract:	prostate
esophagus	salivary gland
stomach	sciatic nerve
duodenum	skeletal muscle (thigh)
jejunum	skin
ileum	spinal cord
cecum	spleen
colon	sternum
rectum	thymus
ovary	thyroid/parathyroid
testes with epididymis	trachea
heart	uterus
urinary bladder	
gross lesions	

d. Histopathology examination:

A full complement of organs and tissues consisted of the following:

adrenals	lung with bronchi
bone & bone marrow	liver
kidneys	lymph nodes (tracheobronchial and mesenteric)
ovary	
testis with epididymis	pancreas
heart	pituitary
spinal cord (entire)	spleen
thymic region	thyroid/parathyroid

A grading system for any lesion consisting of trace, mild, moderate, and severe was used to define gradable lesions for comparison purposes.

7. Statistics: Statistical analysis methods were not reported since there were only 2 dogs per dose group.

8. Quality assurance: A statement of no data confidentiality claim, a statement of compliance, and a quality assurance statement were signed and included in the report.

Results

1. Clinical observation: Ptyalism was seen in all DEET treated male dogs, and the severity and frequency showed a dose-response relationship (Table 1, page 9). Ptyalism seen in 50 mg/kg males was slight with a rather low frequency (once for one male and twice for the other). In female dogs, ptyalism was only seen in 100 and 400 mg/kg groups, and it was graded as slight and with low frequency (once) in 100 mg/kg group. No ptyalism was observed in any control dogs. Abnormal head movements were also seen in the two 400 mg/kg males.

   Relaxed nictitating membrane was seen in essentially all groups, and it was graded as slight. Emesis was also observed in either one or two dogs of all groups (Table 1). The finding of emesis and relaxed nictitating membrane did not appeared to be compound related.

2. Survival rates: No deaths occurred during the study.

3. Physical examination: The physical examination did not revealed a compound-related effect in any group of the treated dogs.

4. Body weights: The mean body weight values were excerpted from the report and presented in Table 2. In males, the body weights measured at 8 weeks were comparable between the treated and the controls. However, in comparison of the 8-week body weight of each dose level to that of the pretest and expressed as percentage difference from the pretest, there was a decrease in this value in 400 mg/kg males and females relative to that of the corresponding controls. This decrease reflected a decrease in body weight gains. The body weights of animals in 50, 100, and 200 mg/kg groups were comparable to those of the controls (Table 2).

Table 2+. Mean Body Weights (kg)

Dose Levels (mg/kg)	Males		Females	
	Pretest	8-weeks	Pretest	8-Weeks
0 (Cont.)	8.2	9.7 (18)	8.4	10.0 (19)
50	8.9	11.0 (23)	8.2	9.6 (17)
100	8.7	10.3 (18)	7.5	8.6 (15)
200	8.8	10.6 (21)	8.2	9.8 (20)
400	8.5	9.4 (11)	7.8	7.6 (-3)

+: Data excerpted from the report; p.17 & 33-40 (MRID No.43514201).
( ): % difference from the pretest.
Cont: Control

5. Food consumption: There was a substantial decrease in food consumption in 400 mg/kg females (≈47% decrease) relative to the controls (Table 3). The individual animal data showed both female dogs had reduced food intake beginning at the first day of treatment. The food consumption was also decreased in the lower dose groups but a dose-response relationship was not seen. The food consumption in treated males at dose levels less or equal to 200 mg/kg was increased. There was also a slight decrease in food consumption in 400 mg/kg males (Table 3, page 7).

7. Hematology: The hematological data did not demonstrate any compound-related changes.

8. Clinical chemistry: There was a noticeable decrease (≈29%) in cholesterol levels in 400 mg/kg males at the terminal sacrifice (Table 4; page 13). The decrease in cholesterol levels were seen in all dose groups of males, but in 200 mg/kg or lower dose levels, the decrease was within the range of the cholesterol level of the controls (males). The reduced

cholesterol levels in treated males appeared to be dose-related. Other clinical chemical parameters were comparable between the treated and the control dogs.

Table 3+: Average Food Consumption for 8 Weeks of Study

Dose Levels (mg/kg/day)	Average Food Consumption (g/dog/day)[a] (% difference from the controls)	
	Males	Females
0 (Control)	288	340
50	355 (23)	287 (-16)
100	311 (8)	259 (-24)
200	306 (6)	319 (- 6)
400	269 (-7)	181 (-47)

+: Data excerpted from the report; p.18 (MRID No.43514201).

a: These values were calculated from the mean weekly food consumption data.

8. Macroscopic: The gross examination did not reveal any compound-related effects.

9. Organ weights: The relevant organ weights were excerpted from the report and presented in Table 5. There was a bilateral decrease (9-23%) in the absolute testis/epididymis weight of 400 mg/kg males. A decrease in brain weight was seen in 400 mg/kg males, but a dose-response relationship was not present.

Table 5+
Summary of Selective Organ Weights in Male Dogs

mg/kg	Brain (g)	Testis with epididymis	
		Left	Right
(Control) 0	80.03	8.72	8.16
50	76.50	9.83	8.57
100	71.13	8.97	8.43
200	78.31	8.87	6.33
400	70.12	7.94	6.26

+: Data excerpted from the report; p. 63-73 (MRID No. 43514201).

10. Histopathology: Compound-related histological changes were not found (Table 6, page 14).

Discussion

Groups of beagle dogs (2/sex/dose) received DEET in a gelatin capsule at dose levels of 50, 100, 200, or 400 mg/kg/day. The control animals received white mineral oil in gelatin capsule. Each daily dose was divided into two equal administrations. One was administered in the morning, and other was given in the afternoom at one hour following the presentation of the food.

The clinical observations data showed an increase in ptyalism in all treated males and in 100 mg/kg or above females and an increase in abnormal head movements in 400 mg/kg males. The finding of ptyaliam in 50 mg/kg males was graded as slight and occurred only once in one dog and twice in the other. In the absence of other clinical signs at this dose level and the fact that ptyalism was not seen in 50 mg/kg females, the toxicological significance of ptyalism at 50 mg/kg males is uncertain. However, at doses higher than 50 mg/kg, the frequency of occurrence and severity of ptyalism were greater with increasing dose level in both males and females.

Relative to the controls, there was a decrease in body weight gains in 400 mg/kg males and females, and that in female dogs was more marked. Food consumption was substantially reduced in 400 mg/kg females.

There was a decrease in cholesterol level in 400 mg/kg male dogs. A decrease in testis/epididymis weight was found in 400 mg/kg males. However, both gross examination and histopathology did not any changed in the testis or any other organs.

The reliability of the results of this study is compromised by the small no of dogs (2/sex/dose) used, and a useful NOEL and LEL could not be established.

Based on the above results, the registrant selected 400 mg/kg as the highest dose and 30 and 100 mg/kg as low and mid dose, respectively, for a chronic toxicity study in dogs. The selected doses for the chronic toxicity appeared to be adequate.

This study is classified as **supplementary**, and does not meet the data requirements for a subchronic oral toxicity study in dogs (82-1).

Table I+

Individual Clinical Signs
Male

Group, Dog Number		Week of Study Onset - Duration		Frequency
0 mg/kg/day (Control):				
3006	Thin, slight	8	8	1
	Soft stool, moderate	1	9	3
	Soft stool, slight-moderate	3	8	4
	Soft stool, slight	4	4	1
	Soft stool, slight-marked	7	7	1
	Diarrhea, slight	7	7	1
	Papillomas, mouth	1	2	2
	Excessive lacrimation, both eyes, slight	4	4	1
	Excessive lacrimation, left eye, slight	5	6	2
	Excessive lacrimation, right eye, slight	7	7	1
3007	No visible abnormalities	1	6	4
	Soft stool, slight	2	3	2
	Food-like emesis, slight	7	7	1
	Soft stool, slight-moderate	8	8	1
	Scars, right ear, small	8	8	1
	Papillomas, mouth, multiple	7	8	2
50 mg/kg/day:				
3011	Soft stool, moderate	1	7	3
	Soft stool, slight-moderate	2	8	5
	Diarrhea, moderate	2	2	1
	Soft stool, slight	9	9	1
	Abrasion, left forefoot, digit, small	7	7	1
	Papillomas, mouth	1	2	2
	Ptyalism, slight	8	8	2
	Injection of sclera, left eye, slight	1	2	2
	Relaxed nictitating membrane, both eyes, slight	3	8	5
	Injection of sclera, both eyes, slight	8	8	1
3015	No visible abnormalities	3	4	2
	Soft stool, moderate	1	1	1
	Soft stool, slight-moderate	2	2	1
	Soft stool, slight	5	6	2
	Food-like emesis, slight	7	7	1
	Oily coat, slight	6	6	1
	Papillomas, mouth	6	8	3
	Ptyalism, slight	8	8	1
100 mg/kg/day:				
3013	No visible abnormalities	2	2	1
	Soft stool, moderate	1	4	2
	Frothy emesis, moderate	1	1	1
	Soft stool, slight	6	6	1
	Soft stool, slight-moderate	7	8	2
	Ptyalism, slight-moderate	1	3	2
	Ptyalism, slight	4	8	4
	Relaxed nictitating membrane, right eye, slight	3	4	2
3017	No visible abnormalities	7	7	1
	Soft stool, slight-moderate	1	8	2
	Discolored feces, red	1	1	1
	Soft stool, slight	3	3	1
	Soft stool, moderate-marked	4	4	1
	Soft stool, moderate	5	5	1
	Diarrhea, slight	5	5	1
	Scabbed area, right hindfoot, small	2	2	1
	Ptyalism, slight	1	6	3
200 mg/kg/day:				
3008	Soft stool, slight-moderate	1	8	7
	Soft stool, slight-marked	3	3	1
	Diarrhea, slight	8	8	1
	Soft stool, moderate	9	9	1
	Ptyalism, slight	1	1	1
	Papillomas, mouth	2	3	2
	Ptyalism, moderate-marked	2	2	1
	Ptyalism, slight-moderate	3	8	5
	Papillomas, mouth, multiple	4	7	3
	Injection of sclera, left eye, slight	1	7	2
	Injection of sclera, both eyes, slight	2	8	2
	Relaxed nictitating membrane, both eyes, slight	4	4	1
	Injection of sclera, both eyes, moderate	8	8	1
3012	Soft stool, moderate	1	7	3
	Food-like emesis, moderate	1	1	1
	Soft stool, slight-moderate	2	8	3
	Food-like emesis, slight	2	8	2
	Frothy emesis, moderate	2	2	1
	Soft stool, slight	4	4	1
	Diarrhea, slight	5	8	2
	Ptyalism, slight-moderate	1	3	2
	Ptyalism, slight-marked	2	2	1
	Ptyalism, moderate	5	5	1
	Ptyalism, slight	6	8	2
	Relaxed nictitating membrane, both eyes, slight	2	8	7

555-027

Onset = Week first observed
Duration = Week last observed
Frequency = Number of weeks observed

+: Data excerpted from the report, p. 23-32 (MRID No. 43514201).

Table 1 Cont.

Individual Clinical Signs
Male

Group, Dog Number		Week of Study Onset - Duration	Frequency
400 mg/kg/day:			
3009	Abnormal head movements, slight-moderate	1 - 1	1
	Abnormal head movements, moderate	2 - 2	1
	Soft stool, slight-moderate	1 - 3	2
	Food-like emesis, slight-marked	1 - 1	1
	Soft stool, moderate	2 - 2	1
	Food-like emesis, moderate	7 - 9	2
	Soft stool, slight	8 - 8	1
	Food-like emesis, slight-moderate	8 - 8	1
	Erythema, left ear, moderate	6 - 6	1
	Erythema, right ear moderate	7 - 7	1
	Erythema, right ear, slight	8 - 8	1
	Erythema, ear flap, left, slight	8 - 8	1
	Ptyalism, slight-marked	1 - 8	4
	Ptyalism, moderate-marked	2 - 2	1
	Ptyalism, moderate	3 - 4	2
	Ptyalism, slight-moderate	6 - 6	1
	Relaxed nictitating membrane, both eyes, slight	1 - 8	8
	Injection of sclera, both eyes, slight	6 - 8	2
3014	Abnormal head movements, slight-moderate	1 - 1	1
	Food-like emesis, moderate-marked	1 - 1	1
	Frothy emesis, moderate	1 - 1	1
	Soft stool, moderate	2 - 7	2
	Food-like emesis, marked	2 - 6	2
	Soft stool, moderate-marked	4 - 4	1
	Food-like emesis, slight-moderate	5 - 5	1
	Ptyalism, slight-marked	1 - 3	3
	Ptyalism, slight-moderate	4 - 8	4
	Ptyalism, slight	6 - 6	1
	Excessive lacrimation, left eye, slight	2 - 3	2
	Injection of sclera, left eye, slight	3 - 3	1
	Relaxed nictitating membrane, both eyes, slight	3 - 8	6
	Injection of sclera, both eyes, slight	7 - 7	1

Onset = Week first observed
Duration = Week last observed
Frequency = Number of weeks observed

Table 1 Cont.

Individual Clinical Signs
Female

Group, Dog Number		Week of Study Onset	Duration	Frequency
0 mg/kg/day (Control):				
3019	Thin, slight	5	5	1
	Soft stool, slight-marked	1	7	3
	Soft stool, slight-moderate	2	8	3
	Diarrhea, moderate	2	5	3
	Diarrhea, slight	4	4	1
	Soft stool, slight	6	9	2
	Food-like emesis, slight	9	9	1
	Ocular discharge, right eye, mucoid, moderate	1	1	1
	Injection of sclera, right eye, slight	1	1	1
	Relaxed nictitating membrane, both eyes, slight	3	8	4
	Injection of sclera, both eyes, slight	8	8	1
3022	Soft stool, slight-moderate	1	8	5
	Diarrhea, moderate	2	7	2
	Soft stool, marked	3	3	1
	Soft stool, moderate-marked	4	4	1
	Soft stool, slight	5	5	1
	Diarrhea, slight	5	6	2
	Food-like emesis, slight	6	6	1
	Scars, ear flap, left, multiple, small	3	8	6
	Excessive lacrimation, right eye, slight	4	7	2
50 mg/kg/day:				
3020	Soft stool, slight-moderate	1	8	4
	Diarrhea, slight-moderate	1	1	1
	Soft stool, slight	2	6	2
	Diarrhea, slight	3	7	2
	Soft stool, slight-marked	7	7	1
	Excessive lacrimation, both eyes, slight	3	7	4
	Excessive lacrimation, both eyes, moderate	4	4	1
	Excessive lacrimation, right eye, slight	8	8	1
3021	Tail bent, distal	3	8	6
	Soft stool, slight-moderate	1	5	4
	Diarrhea, slight-moderate	3	3	1
	Diarrhea, moderate-marked	4	4	1
	Soft stool, moderate	6	8	2
	Soft stool, slight-marked	7	7	1
	Vaginal discharge, green, yellow, slight	8	8	1
	Ocular discharge, left eye, mucoid, slight	1	4	3
	Injection of sclera, left eye, slight	1	5	4
	Ocular discharge, left eye, mucoid, marked	3	3	1
	Injection of sclera, left eye, moderate	4	6	2
	Relaxed nictitating membrane, left eye, slight	4	4	1
	Ocular discharge, right eye, mucoid, slight	5	5	1
	Relaxed nictitating membrane, both eyes, slight	6	8	3
	Ocular discharge, left eye, mucoid, moderate	7	7	1
	Injection of sclera, left eye, marked	7	7	1
	Ocular discharge, both eyes, mucoid, slight	8	8	1
	Injection of sclera, both eyes, slight	8	8	1
100 mg/kg/day:				
3018	Soft stool, slight-marked	1	7	2
	Discolored feces, red	1	7	2
	Mucoid diarrhea, marked	1	1	1
	Soft stool, moderate	2	4	2
	Soft stool, slight-moderate	3	3	1
	Emesis, red, material, mucoid, moderate	4	4	1
	Mucoid diarrhea, moderate	5	7	2
	Soft stool, slight	6	9	3
	Mucoid diarrhea, red, marked	6	6	1
	Diarrhea, slight-moderate	6	6	1
	Diarrhea, marked	7	7	1
	Food-like emesis, moderate	8	8	1
	Ptyalism, slight	1	1	1
3028	No visible abnormalities	3	3	1
	Soft stool, slight-moderate	1	8	3
	Mucoid diarrhea, moderate	1	1	1
	Soft stool, slight	5	7	2
	Soft stool, moderate	6	6	1
	Ptyalism, moderate	1	4	2
	Ptyalism, slight	2	2	1

555-027

Onset = Week first observed
Duration = Week last observed
Frequency = Number of weeks observed

Table 1 Cont.

Individual Clinical Signs
Female

Group, Dog Number		Week of Study Onset - Duration		Frequency
200 mg/kg/day:				
3023	Soft stool, moderate	1	1	1
	Frothy emesis, moderate	1	1	1
	Diarrhea, slight-moderate	1	1	1
	Soft stool, slight-moderate	2	8	5
	Diarrhea, slight	2	2	1
	Soft stool, slight	3	9	3
	Papillomas, mouth	1	2	2
	Ptyalism, moderate	1	3	3
	Excessive lacrimation, left eye, slight	1	7	6
	Injection of sclera, both eyes, slight	1	8	6
	Excessive lacrimation, both eyes, slight	6	8	2
3029	Soft stool, slight-moderate	1	8	6
	Food-like emesis, moderate	1	1	1
	Diarrhea, slight	1	8	3
	Soft stool, slight	2	2	1
	Diarrhea, slight-moderate	2	2	1
	Soft stool, slight-marked	4	4	1
	Diarrhea, marked	5	5	1
	Soft stool, moderate	9	9	1
	Ptyalism, slight-moderate	1	5	2
	Ptyalism, slight-marked	2	2	1
	Ptyalism, moderate	4	4	1
	Ptyalism, slight	6	8	3
	Papillomas, mouth	7	8	2
	Excessive lacrimation, both eyes, slight	2	6	3
	Excessive lacrimation, left eye, moderate	5	7	2
	Excessive lacrimation, right eye, slight	5	7	2
	Excessive lacrimation, both eyes, moderate	8	8	1
	Pupils dilated, both eyes, moderate	8	8	1
400 mg/kg/day:				
3025	Decreased activity[a]	1	3	2
	Abnormal head movements, slight-marked	1	1	1
	Trembling, moderate	3	3	1
	Decreased activity, slight	3	3	1
	Abnormal head movements, moderate	5	5	1
	Food-like emesis, moderate	1	2	2
	Discolored feces, red	2	2	1
	Mucoid diarrhea, moderate	2	2	1
	Diarrhea, slight-moderate	2	2	1
	Emesis, red, material, mucoid, moderate	4	4	1
	Frothy emesis, moderate	8	8	1
	Food-like emesis, slight	9	9	1
	Ptyalism, slight-marked	1	7	3
	Tooth problems	3	8	6
	Ptyalism, moderate-marked	3	3	1
	Ptyalism, moderate	4	4	1
	Ptyalism, slight-moderate	5	5	1
	Ptyalism, slight	6	8	2
	Injection of sclera, left eye, slight	5	5	1
3026	Trembling[a]	1	1	1
	Food-like emesis, moderate-marked	1	1	1
	Frothy emesis, marked	1	1	1
	Diarrhea, slight	1	8	2
	Soft stool, slight-moderate	2	8	3
	Mucoid diarrhea, moderate-marked	2	2	1
	Food-like emesis, slight	2	2	1
	Diarrhea, moderate	2	2	1
	Food-like emesis, moderate	4	5	2
	Soft stool, slight	5	5	1
	Diarrhea, slight-moderate	5	5	1
	Soft stool, moderate	6	6	1
	Ptyalism, slight-marked	1	2	2
	Ptyalism, slight-moderate	3	6	2
	Ptyalism, moderate	4	5	2
	Ptyalism, slight	7	8	2

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Onset = Week first observed
Duration = Week last observed
Frequency = Number of weeks observed
a = Severity inadvertently not recorded

Table 4+

Males: Summary of Biochemical Values

Parameters Measured	WEEK OF STUDY	0 mg/kg/day (Control) MEAN	S.D.	N	50 mg/kg/day MEAN	S.D.	N	100 mg/kg/day MEAN	S.D.	N	200 mg/kg/day MEAN	S.D.	N	400 mg/kg/day MEAN	S.D.	N
Creatine Phosphokinase IU/l	Pretest	202	31.8	2	298	118.8	2	402	58.7	2	367	199.4	2	262	42.4	2
	Terminal	166	4.9	2	208	101.1	2	239	106.1	2	260	91.2	2	153	29.0	2
Urea Nitrogen mg/dl	Pretest	16	1.4	2	18	2.1	2	17	2.1	2	21	5.7	2	14	2.8	2
	Terminal	18	2.8	2	18	6.4	2	18	7.1	2	18	2.8	2	17	0.7	2
Creatinine mg/dl	Pretest	0.9	0.00	2	0.9	0.07	2	0.8	0.07	2	0.9	0.21	2	0.7	0.14	2
	Terminal	0.8	0.00	2	0.9	0.07	2	0.8	0.07	2	0.9	0.07	2	0.9	0.07	2
Total Protein g/dl	Pretest	5.7	0.21	2	5.4	0.07	2	5.5	0.28	2	5.4	0.14	2	5.2	0.00	2
	Terminal	5.9	0.21	2	5.4	0.14	2	5.4	0.21	2	5.4	0.21	2	5.7	0.07	2
Albumin g/dl	Pretest	2.8	0.00	2	2.8	0.21	2	2.9	0.00	2	2.8	0.14	2	2.8	0.14	2
	Terminal	2.8	0.21	2	2.8	0.28	2	2.9	0.07	2	2.7	0.00	2	3.1	0.00	2
Globulin g/dl	Pretest	2.9	0.21	2	2.6	0.14	2	2.6	0.28	2	2.6	0.00	2	2.4	0.14	2
	Terminal	3.1	0.00	2	2.6	0.42	2	2.5	0.14	2	2.7	0.21	2	2.6	0.07	2
Cholesterol mg/dl	Pretest	179	42.4	2	180	49.5	2	204	0.7	2	186	14.1	2	149	6.4	2
	Terminal	180	56.6	2	174	48.1	2	157	20.5	2	141	18.4	2	128	9.9	2
Glucose mg/dl	Pretest	109	0.7	2	101	7.8	2	102	2.8	2	113	3.5	2	114	2.8	2
	Terminal	113	4.9	2	104	14.1	2	109	10.6	2	109	10.6	2	114	0.7	2

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S.D. Standard Deviation
N - Number of Animals

+: Data excerpted from the report, p. 55 & 56 (MRID No. 43514201).

Table 6. Cont.

Incidence of Microscopic Observations
Terminal Sacrifice: Dogs
Female

TISSUE / OBSERVATION		0 mg/kg/day (Control)		50 mg/kg/day		100 mg/kg/day		200 mg/kg/day		400 mg/kg/day	
		DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Adrenal, Cortex		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Adrenal, Medulla		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Bone Marrow, Rib		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Bone, Rib		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Eye		(0)	(1)	(0)	(1)	(0)	(0)	(0)	(1)	(0)	(0)
Within normal limits		0	1	0	1	0	0	0	1	0	0
Heart		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Kidney		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	1	0	0	0	0	0	0	0	0
Mineralization,		0	1	0	2	0	2	0	2	0	2
	-trace	0	1	0	2	0	1	0	1	0	2
	-mild	0	0	0	0	0	1	0	1	0	0
Liver		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	1	0	2	0	0	0	2	0	0
Inflammation, trace		0	1	0	0	0	0	0	0	0	0
Lymphocytic infiltration, trace		0	0	0	0	0	1	0	0	0	1
Vacuolar change, mild		0	0	0	0	0	1	0	0	0	1
Lung		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	0	0	0	0	1	0	1	0	1
Interstitial pneumonia,		0	2	0	2	0	1	0	1	0	1
	-trace	0	1	0	2	0	0	0	0	0	0
	-mild	0	0	0	0	0	0	0	1	0	1
	-moderate	0	1	0	0	0	1	0	0	0	0
Lymph Node, Mesenteric		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Lymph Node, Tracheobronchial		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Oral Tissues		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Lymphoid hyperplasia, moderate		0	0	0	0	0	0	0	0	0	1
Ovary		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	1	0	2	0	2	0	2	0	1
Mineralization, trace		0	1	0	0	0	0	0	0	0	1
Pancreas		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2
Parathyroid		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	1	0	1	0	2	0	1
Cyst,		0	0	0	1	0	1	0	0	0	1
	-trace	0	0	0	1	0	0	0	0	0	0
	-mild	0	0	0	0	0	1	0	0	0	1
Pituitary		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	1	0	1	0	1	0	1	0	0
Cyst,		0	1	0	1	0	1	0	1	0	2
	-trace	0	1	0	0	0	1	0	1	0	1
	-mild	0	0	0	1	0	0	0	0	0	1
Spleen		(0)	(1)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	1	0	1	0	2	0	2	0	2
Fibrosis, mild		0	0	0	1	0	0	0	0	0	0
Pigment, brown, trace		0	0	0	1	0	0	0	0	0	0
Thymus		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	1
Atrophy, moderate		0	0	0	0	0	0	0	0	0	1
Thyroid		(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits		0	2	0	2	0	2	0	2	0	2

CODE: () = NUMBER OF ANIMALS EXAMINED

TABLE 6+.

Incidence of Microscopic Observations
Terminal Sacrifice: Dogs
Male

011625

TISSUE OBSERVATION	0 mg/kg/day (Control) DOS	0 mg/kg/day (Control) SAC	50 mg/kg/day DOS	50 mg/kg/day SAC	100 mg/kg/day DOS	100 mg/kg/day SAC	200 mg/kg/day DOS	200 mg/kg/day SAC	400 mg/kg/day DOS	400 mg/kg/day SAC
Adrenal, Cortex	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Adrenal, Medulla	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Bone Marrow, Rib	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Bone, Rib	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Epididymis	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Eye	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(1)	(0)	(1)
Within normal limits	0	0	0	1	0	0	0	1	0	1
Heart	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	1	0	2	0	2	0	2
Mineralization, trace	0	0	0	1	0	0	0	0	0	0
Kidney	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	0	0	1	0	0	0	0	0	0
Lymphocytic infiltration, trace	0	0	0	0	0	0	0	0	0	1
Mineralization, trace	0	2	0	1	0	2	0	2	0	2
Liver	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	1	0	2	0	1	0	1	0	1
Inflammation, acute, trace	0	0	0	0	0	0	0	0	0	1
Lymphocytic infiltration, trace	0	1	0	0	0	1	0	1	0	0
Lung	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	0	0	2	0	1	0	1
Inflammation, chronic, mild	0	0	0	0	0	0	0	0	0	1
Interstitial pneumonia, trace	0	0	0	2	0	0	0	1	0	0
Mineralization, trace	0	0	0	0	0	0	0	0	0	1
Lymph Node, Mesenteric	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Lymph Node, Tracheobronchial	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Oral Tissues	(0)	(1)	(0)	(1)	(0)	(0)	(0)	(2)	(0)	(0)
Within normal limits	0	0	0	1	0	0	0	2	0	0
Papilloma	0	1	0	0	0	0	0	0	0	0
Pancreas	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Parathyroid	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(1)
Within normal limits	0	2	0	2	0	1	0	1	0	1
Cyst, trace	0	0	0	0	0	0	0	1	0	0
Fatty infiltration, mild	0	0	0	0	0	1	0	0	0	0
Pituitary	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	0	0	2
Cyst,	0	0	0	0	0	0	0	2	0	0
-trace	0	0	0	0	0	0	0	1	0	0
-mild	0	0	0	0	0	0	0	1	0	0
Skeletal Muscle	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within normal limits	0	1	0	0	0	0	0	0	0	0
Spleen	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Stomach	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
Within normal limits	0	0	0	0	0	0	0	1	0	0
Testis	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	2
Thymus	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	2	0	2	0	1
Atrophy, mild	0	0	0	0	0	0	0	0	0	1
Thyroid	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)
Within normal limits	0	2	0	2	0	1	0	2	0	2
Mineralization, trace	0	0	0	0	0	1	0	0	0	0

555-027

CODE: () = NUMBER OF ANIMALS EXAMINED

+: Data excerpted from the report, p. 86-91 (MRID No. 43514201).

13544

011826

Chemical:	**N,N-Diethyl-meta-toluamide and other iso**
PC Code:	**080301**
HED File Code	**13000 Tox Reviews**
Memo Date:	**08/04/95**
File ID:	**TX011625**
Accession Number:	***412-02-0004***

HED Records Reference Center
10/01/2001